Chemical Mixtures Research: Significance and Future Perspectives

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The study of chemical mixtures is an important area of research because of its potential impact on how risk is assessed in populations exposed to multiple environmental agents. Clearly, individuals are exposed to myriad chemical agents during their lifetime. Low-dose chronic exposure to environmental agents and the additional lifestyle factors that may affect health are extremely difficult to assess by conventional approaches. Classic scientific approaches, while serving the research community well in assessing the impact of single chemicals on the functioning of biological systems, may be inadequate to address aggregate and cumulative exposures of multiple chemicals in living systems. Over the past decades, there have been extraordinary scientific and computational technology advances. Through the incorporation of technological advances in microarrays, laser capture microdissection, imaging, and high through-put technologies, we can now study in greater detail the properties of mixtures and begin to define those characteristics that are sufficiently similar to allow extrapolation of data from one mixture to another. Bioinformatics and other computational approaches would also be integrated into this research scheme, not only for data analysis but also to develop predictive models that could be used for risk assessment. Integrated approaches that address these issues will be necessary to advance our understanding of the health relevance of exposure to mixtures. ***Key words:*** **biotechnology, chemical mixtures, computer modeling, interdisciplinary collaboration, mechanistic toxicology, nanotechnology.** ***Environ Health Perspect*** **110(suppl 6):891–892 (2002).**
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In the spirit of interdisciplinary collaboration beyond biology, the conference "Application of Technology to Chemical Mixture Research" was held at the Colorado State University in Fort Collins, Colorado, in January 2001 under the joint sponsorship of the National Institute of Environmental Health Sciences (NIEHS) Superfund Basic Research Program (SBRP) and Colorado State University. Thirty-one invited speeches and 22 poster presentations covered the following areas of technology in relation to chemical mixture research: cell and molecular methodologies, computer and statistical modeling, engineering and chemical technologies, and nanotechnology. Most of the highlights are captured in this monograph.

This introduction, the opening commentary of the monograph, provides *a*) an overview of the significance of chemical mixture research; *b*) a research framework in light of the recent advances in technology including biotechnology; *c*) an emphasis of the importance and necessity of interdisciplinary collaboration, particularly beyond the traditional boundaries; and *d*) future perspectives, including "thinking outside the box."

Significance of Chemical Mixture Research

The mission of NIEHS is to reduce the burden of human illness and dysfunction from environmental exposures to chemical agents by understanding the interactions between environmental exposure, individual susceptibility, and time. Historically, toxicity and carcinogenicity testing, as well as mechanistic research on environmental chemicals, has focused on single agents. Over the years this approach on environmental chemicals has been critical in providing information that has led to a better understanding of the interactions of exposure and susceptibility in relation to time. Indeed, the setting of standards for single substances on the basis of our acquired knowledge base has been accepted as an important and generally accepted tool in the protection of human health. However, it is becoming increasingly recognized that humans are not exposed to single chemicals. Rather, humans are exposed either concurrently or sequentially, by various routes of exposure, to a larger number of chemicals from a variety of sources over varying periods of time.

The magnitude of the problem is immense. In our daily living, exposures to mixtures of chemicals are ubiquitous in the air we breathe, the food we eat, and the water we drink. For example, if we consider hazardous waste, it has been estimated that 275 million tons of hazardous wastes are produced on an annual basis in the United States and for municipal solid waste, 1,900 lb/person/year is produced. There are currently about 39,925 sites on the U.S. Environmental Protection Agency (U.S. EPA) inventory of uncontrolled waste sites. Those posing the most significant threat to public health and the environment have been designated to the National Priority List [NPL (U.S. EPA 2002)] for cleanup. More than 2,000 unique substances have been identified in the environmental media of sites sampled by the U.S. EPA during its site characterization studies and typically mixes of hazardous substances are numbered in the hundreds. The National Research Council, using U.S. EPA data, has estimated that 41 million people live within a 4-mile radius of 1,134 NPL sites (U.S. EPA 2002).

Toxicity studies of mixtures have been ongoing for some decades. In earlier years, most of the studies concerned binary mixtures. Later studies with defined mixtures of more than two compounds and studies with complex mixtures were also reported. However, to design a full factorial study to examine the interaction(s) of three chemicals at five different dose levels requires 125 treatment groups and 750 animals if six animals are to be included in each treatment group. The result of such a large expenditure of time and funds would be information on one temporal sequence of exposures at one time point postexposure. Clearly, the information gained would be of limited value. Therefore, it would seem prudent to develop a rational approach to studying mixtures by prioritizing and identifying chemical mixtures that are based on known human exposures and developing innovative experimental, mechanistic, and statistical strategies.

A rational approach to identifying chemicals and chemical mixtures on the basis of credible exposure assessment is essential to advance our knowledge on mixtures. The National

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Toxicology Program (NTP) at NIEHS in collaboration with the Centers of Disease Control and Prevention is developing a strategy to quantify the human body burden of environmental chemicals. The Agency for Toxic Substances and Disease Registry has developed HazDat, a hazardous substance release/health effects database (*http://www.atsdr.cdc.gov/hazdat.html*). Data evaluated from all public health assessments have allowed investigators to identify several classes of contaminants most frequently reported, including volatile organic compounds, inorganic substances such as metals, halogenated pesticides, polyaromatic hydrocarbons, phenols/phenoxy acids, phthalates, nitrosamines/ethers/alcohols, and organophosphates. Analysis of this database has identified preliminarily 10 most frequently found binary combinations of chemicals at wastes sites. For soil, metal/metal combinations are most frequently encountered, whereas in water, metal/metal, metal/organic and organic/organic combinations are seen. Review of these and other data may provide quidance in setting priorities for chemical mixtures to be studied.

Potential Future Development

Innovative and credible strategies for developing experimental designs and statistical, computational, and predictive approaches need to be encouraged. Below are three examples of broad areas of priority research that may yield fruitful and useful results on chemical mixture toxicology.

First, the interaction between environmental exposures and genes with respect to time is the central axiom for environmental health research at NIEHS. Judith Stern, University of California, Davis, has stated succinctly: "... Genetics loads the gun, but environment pulls the trigger. . . " The revolution of genomics and bioinformatics prompts us to ask: Do specific toxicants have unique signatures? Do different cells and tissues exhibit different responses or signatures? Do different species exhibit overlapping patterns of gene responses? Is the toxicant signature pattern altered with age or stages of development? Is gene expression a useful approach for investigating the toxicities of chemical mixtures? Is single nucleotide polymorphism even more important in toxicological interactions of chemical mixtures?

Second, understanding the mechanistic bases of interactions at the quantitative level is necessary before confidence can be gained to provide realistic risk assessments for chemical mixtures. For example, integrating physiologically based pharmacokinetic/pharmacodynamic modeling, biologically based dose–response modeling, and reaction network modeling with innovative experimental approaches, the mechanism for nonadditive interactions of multiple chemicals could be discerned and extrapolated to environmentally relevant concentrations of chemicals. If we consider toxicities as perturbations of homeostasis beyond the normal physiological ranges, computer modeling of toxicities, including chemical mixture toxicities, simplifies to changing modeling parameters of normal biological systems based on mechanistic understanding. Thus, the basic, normal biological processes must be understood first and then the mechanistic basis for cellular and/or molecular perturbations and associated adverse health effects. It should be noted that we do not need to know every detail in order to develop a useful computer modeling tool. To quote J.E. Bailey, an eminent biochemical engineer, before his untimely death in May 2001:

> . . . Qualitative and quantitative understanding and corresponding methodologies for designing desired properties of many complex systems have been successfully achieved in the fields of chemistry, physics, and the associated engineering disciplines without knowing all aspects of systems structure and certainly without knowing all parameter values involved. The same must be possible for biology. . . . (Bailey 2001)

With the mining of biochemical and physiological data from the past hundred years, there is great promise in the development of such "*in silico* toxicology."

Third, the development of high-throughput technologies for carcinogenicity and toxicity testing is important. Considering the approximately 80,000 chemicals in commerce (NTP 2002), the task of testing these chemicals on an individual basis, let alone as a mixture, is not feasible. In line with the series of questions posed in the first example above, is it possible that the integration of short-term, focused, mechanistic toxicology with some or all of the recent advances of technology and biotechnology (i.e., genomics, proteomics, bioinformatics, computer modeling, robotics, and nanotechnology) may provide high-throughput and scientifically credible bioassays for carcinogenicity and other toxicities?

Interdisciplinary Collaboration

Why is interdisciplinary collaboration important? In an AAAS Plenary Lecture on 13 February 1998, Dr. Harold Varmus, then Director of the National Institutes of Health, emphasized, among others, two specific themes: ". . . Discoveries in biology and medicine depend on progress in many fields of science. . . ." and ". . . Methods that dramatically expand biological data also demand new modes of analysis and new ways to ask scientific questions. . . ." He said: ". . . In short, biology is not only for biologists. . . ." Scientific developments are increasingly dependent on cross-fertilization from different disciplines. The NIEHS SBRP is one such example where biomedical researchers work together with ecologists, engineers, etc., to allow synergistic creativity in addressing the complex problems of Superfund hazardous waste disposal sites. Today, the world grows closer because of advances in telecommunication technologies. As a result, investigators have ready access to a wealth of information that was unavailable to them previously. Because of the complexity of problems related to toxicology research on chemical mixtures, it is fruitful to open our minds to the potential utilization of concepts and technologies developed in other disciplines. Two articles (Laio et al. 2002; Klein et al. 2002) in this monograph provide an example of such interdisciplinary research collaboration.

Thinking Outside the Box

Parallel to the thinking that led to the present-day interest and research activities in chemical mixture research, we cannot help but realize that chemicals, including mixtures, are but one of many factors that may cause injuries to our body. The primary consideration in toxicology is the perturbation of homeostasis to the point of cellular, organ, or bodily injury by the culmination of chemical, biological, physical agents, and even psychological stress, within a window of opportunity—the exposure period to all the stressors. Thus, we, the toxicologists, should ultimately examine the effects of multiple stressors on cells, organs, and/or organisims in comparison to their respective "ground state"—that is, the normal, naive physiological conditions. In that sense, when the concepts of virtual cells, virtual organs, and virtual humans become a reality, the present concerns of the complexity of chemical mixtures and multiple stressors may no longer be an important issue.

Conclusion

The area of chemical mixture research has come a long way in the last twenty years or so. It is gratifying to see the increasing levels of interest in scientific and the regulatory communities toward chemical mixtures. The two scientific conferences on chemical mixtures and the subsequent publication of two monographs (an earlier monograph [Yang and Suk, 1998] and the present monograph) serve as an important forum for the synergistic creativity in this important yet difficult area of research.

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