

Some Critical Issues and Concerns Related to Research Advances on the Toxicology of Chemical Mixtures

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This paper addresses some of the issues and concerns on research advances on the toxicology of chemical mixtures. Emphases will be selectively given to the following questions and answers: Can mechanistic studies be conducted on chemical mixtures? The fact that any studies, including mechanistic studies, of single chemicals are really the study of the parent chemical plus its metabolites underscores the relevance of mechanistic studies on chemical mixtures. Can predictions be made on the health effects of chemical mixtures? Some successes are already evident in the literature on simpler chemical mixtures. For more complex mixtures, it is possible and we propose an approach here. What can we learn from other disciplines (the importance of interdisciplinary collaboration)? Two aspects, the knowledge and methodologies available in clinical pharmacology and the latest advances in structure-oriented lumping in chemical engineering, are discussed in detail. Unrepeatable results: The possibility of magnification of biologic variability because of low-level exposures to chemical mixtures is suggested with special reference to some known examples, including the controversial study on synergistic interactions of endocrine disruptors. Is the driving force for scientific investigations on chemical mixtures the legislative and regulatory atmosphere? Two laws with chemical mixtures specifically in the language are quoted and discussed. Their implications regarding research funding and activities are described. What are the pitfalls of applying for research funding on investigating chemical mixtures? The dilemma at least one investigator faces in pursuing research funding is elaborated. The questions and issues listed above are not all inclusive, but they represent some of the aspects that need to be brought into the open in the scientific community for discussion and/or debate. Thus, the primary objective of this paper is to provide some momentum for the beginning of a fruitful and stimulating discussion. — *Environ Health Perspect* 106(Suppl 4):1059–1063 (1998). <http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-4/1059-1063yang/abstract.html>

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Introduction

When dealing with Superfund hazardous waste disposal sites, one cannot avoid facing the issues of chemical mixtures. However, the problems of multiple chemical exposures and the related possible health consequences go far beyond Superfund hazardous waste disposal sites. The reality

is that there is really no such thing as a single chemical exposure (1,2). Although all scientists appear to agree that exposure to chemical mixtures is the rule rather than the exception, few would risk their scientific careers to devote themselves to the study of chemical mixtures. It is much

more comfortable and safe to go with the crowd on single-chemical research than to serve as a lightning rod. This fear is justifiable because research funding is unlikely to go to projects that are unusual, risky, and potentially controversial.

The past 15 years or so have witnessed the gradual maturing of the area of toxicology of chemical mixtures. Symposia at annual meetings in major scientific societies include topics related to chemical mixtures with increasing frequency. Major conferences on chemical mixtures are held almost on an annual basis on both sides of the Atlantic Ocean (3). More investigators are getting involved in this area of research, and the research work is becoming more and more sophisticated. As is true with any developing area, there are issues unique to chemical mixtures, and there are concerns facing the investigators engaging in research work on chemical mixtures.

A number of recent publications (1,4,5) have dealt with some of the perennial issues in chemical mixture research, such as: Are there toxicologic interactions at low, environmentally realistic exposure levels to chemical mixtures? How does one go about studying chemical mixtures? What chemical mixtures are to be used in studies? To avoid repetition, these issues are not discussed here. Instead, a number of specific questions are posed and each is addressed in the context of the current development of this area of research.

Commentary on Critical Issues

Can Mechanistic Studies Be Conducted on Chemical Mixtures?

The short answer to this question is “yes?” In reality, when investigators study a single chemical, they are actually studying a fairly complex mixture because of biotransformation and chemical interactions. For instance, in the body, *n*-hexane is metabolized to 2-hexanol, which can be further metabolized to 2,5-hexanediol and 2-hexanone. Both of these metabolites may in turn be further biotransformed to 5-hydroxy-2-hexanone and go on to form 2,5-hexanedione, a neurotoxic agent and a major metabolite in humans (6). Thus, we have at least six chemicals involved in the study of one single chemical, *n*-hexane, and the toxic species is quite far down the line. In the area of carcinogenesis, it is possible for a chemical mixture to contain

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Abbreviations used: hER, human estrogen receptor; NIEHS, National Institute of Environmental Health Sciences; PBPK/PD, physiologically based pharmacokinetic/pharmacodynamic; QSAR, quantitative structure–activity relationship; SOL, structure-oriented lumping.

chemicals possessing initiation, promotion, and progression properties working in concert to bring about neoplastic transformations. To carry this line of reasoning a little further, why can't various components of a chemical mixture perturbate signal transduction pathway, growth factors, genetic material, oncogenes and suppressor genes, cell differentiation, etc. to either enhance or inhibit the potential of carcinogenesis? Therefore, when dealing with a chemical mixture, the mechanisms of toxicity are there; they are more complex and difficult, but not impossible, to study.

Investigators in the area of endocrine disruptors have had an ongoing discussion about chemical mixtures, synergism, and mechanisms (7). The principal stimulant for these discussions came from a publication (8) in which the investigators reported that combinations of weakly estrogenic environmental pollutants ended up with remarkable synergism, as much as a 1600-fold increase in estrogenic activity. Although this paper was retracted (9) because the results could not be repeated in a number of laboratories, scientific deliberations continued. Among the discussions, one school of thought is that the investigation of possible synergistic endocrine disruptive activities of chemical mixtures should be focused on multiple mechanisms rather than a single mechanism (7). This is additional support for the contention that mechanistic studies can be conducted with chemical mixtures. With the availability of many different types of cell culture systems and the recent advances in molecular biology techniques, the study of multiple mechanisms of a given toxicologic end point induced by a chemical mixture is becoming more and more a reality.

Can Predictions Be Made on the Health Effects of Chemical Mixtures?

Many in the toxicology community would probably think that it is impossible to predict the health effects from exposures to a chemical mixture, particularly a complex chemical mixture, because sometimes we have trouble with even two chemicals at a time. To put things in perspective, I would like to quote a passage from *The Making of the Atomic Bomb* (10) about the early development of nuclear physics. The passage was a summary of a speech by Ernest Rutherford, the great British experimental physicist at Cavendish Laboratory, Cambridge University (Cambridge, England), presented 12 September, 1983.

Hope of Transforming Any Atom

What, Lord Rutherford asked in conclusion, were the prospects 20 or 30 years ahead?

High voltages of the order of millions of volts would probably be unnecessary as a means of accelerating the bombarding particles. Transformations might be effected with 30,000 or 70,000 volts.... He believed that we should be able to transform all the elements ultimately.

We might in these processes obtain very much more energy than the proton supplied, but on the average we could not expect to obtain energy in this way. It was a very poor and inefficient way of producing energy, and anyone who looked for a source of power in the transformation of the atoms was talking moonshine.

At that time, Rutherford was referring to his belief that whoever talks about the liberation of atomic energy on an industrial scale is talking foolishly or nonsensically. Of course, later events proved that Rutherford, an eminent physicist, was wrong.

Can predictions be made on the health effects of chemical mixtures? My answer to this question, at the present time, is: likely. Prediction of health effects usually involves some type of mathematical modeling, which may range from the classical compartmental pharmacokinetic modeling to the currently advancing physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling and/or quantitative structure-activity relationship (QSAR) modeling. Some successes of prediction of pharmacokinetic fate, as well as toxicity of simple chemical mixtures, are already evident in the literature (11-22). The toxicologic end points of prediction, for instance, include an interaction threshold (18,19) and acute lethality due to hepatic injuries (20).

For more complex mixtures, lung cancer risks can be predicted reasonably well based on exposure to cigarette smoke, coke oven mains, and roofing tar. This was made possible by many years of research in human epidemiology and experimental toxicology on the mutagenicity, carcinogenicity, and DNA alteration ability of these mixtures (23-32). As for other complex mixtures, the integration of PBPK/PD, QSAR modeling, and lumping analysis (a modeling tool used in the petroleum industry) may formulate a predictive tool for health effects (33). In the 1960s the application of lumping analysis rendered it possible to predict gasoline production based on a few lumps rather than the thousands

of component chemicals of the petroleum (34,35). Thus, even though relatively little is known about the complex mixture of petroleum, a predictive tool was developed and applied from modeling. If the relatively crude lumping analysis can predict some aspects of catalytic cracking of petroleum approximately 30 years ago, why can't we attempt to predict health effects from chemical mixtures through much more sophisticated modeling such as the structure-oriented lumping (SOL) (36,37)?

The Importance of Interdisciplinary Collaboration: What Can We Learn from Other Disciplines?

The knowledge and methodologies available in clinical pharmacology, aquatic toxicology, and the latest advances in SOL in chemical engineering are examples of research areas for us to explore for the advancement of chemical mixture toxicology. Today the world gets closer and closer because of advances in telecommunication technologies. Investigators have ready access to more and more information that was unavailable to them previously. Scientific developments are increasingly dependent on cross-fertilization from different disciplines. The National Institute of Environmental Health Sciences (NIEHS) Superfund Basic Research Program is one such example where biomedical researchers work side by side with ecologists, engineers, etc. to create synergistic creativity toward addressing the complex problems of Superfund hazardous waste disposal sites.

Because of the complexity of problems related to toxicology research on chemical mixtures, it is fruitful to open our minds to reach out for concepts and technologies in other disciplines for possible applications. Pioneering work in the field of medical pharmacology, which is closely related to toxicology, offers many learning opportunities. For simpler chemical mixtures involving two or three components, the vast literature on drug interactions (38-41) provides excellent opportunities for scientific exploitation. We may profit a great deal from the utilization of isobolographic analysis, response-surface methodology, median-effect principles, and other methodologies (42-47). Equally important and stimulating, the contributions of aquatic toxicology in the last two decades have been particularly useful in the advancement of the toxicology of chemical mixtures for three reasons: aquatic organisms offer alternative experimental models for complex study designs for chemical

mixtures; mixture toxicology has been a major regulatory and research concern in aquatic toxicology since the 1980s; and the advancement of QSAR modeling in relation to aquatic toxicology provides unique opportunities for learning (48–51).

To reach out a little further into the domain of petroleum engineering, there are techniques we may borrow and utilize to address the toxicology of complex chemical mixtures. As indicated previously, the concept of lumping analysis was advanced in the 1960s to provide a predictive modeling tool in petroleum engineering (34,35). Verhaar et al. (33) discussed lumping analysis tailored for toxicologists. The basic principle of lumping analysis was to simplify the thousands of components by lumping similar chemicals together based on boiling points and/or the total carbon numbers of the molecules. In doing so, a complex chemical mixture is made manageable by grouping the thousands of components into a few lumps or a few pseudocomponents. The modeling of this new mixture of a few pseudocomponents, though crude, was useful enough to predict gasoline yields. However, this type of approach neither incorporates realistic process chemistry nor allows for the simultaneous calculation of many properties. Thus, it is not able to predict gasoline composition or how composition impacts the required quality specifications. This area remained unchanged for more than 25 years until a new approach, SOL, emerged. SOL was developed in response to the need for incorporating molecular detail in petroleum chemistry to predict product compositions and properties (36,37). The basic concept of the SOL approach is that any petroleum molecule can be described and represented by a set of structural features or groups (36,37). Huge numbers of molecular structures and their related process chemistry are digitized in a systematic manner such that computer modeling and simulation are possible for complex mixtures. The end results are a much more accurate and powerful predictive capability for both the unknown components and/or the end points of interest such as boiling point, specific gravity, and absolute viscosity of homologous series of petroleum chemicals (36,37).

This powerful tool should be directly applicable to the health effects of complex chemical mixtures because what happens in the body when animals or humans are exposed to complex chemical mixtures is really a manifestation of chemical and

physical processes. Our laboratory suggested an approach to study chemical mixtures by integrating QSAR, lumping analysis, and PBPK/PD modeling (33). We are currently exploring collaborative research opportunities in the application of SOL in the toxicology of chemical mixtures.

Unrepeatable Results: Magnification of Biologic Variability in Chemical Mixtures and at Lower Doses?

In the last 2 years or so, one of the most controversial publications in toxicology was the report by Arnold et al. (8) on highly synergistic activities when weakly estrogenic chemicals are combined and exposed to a yeast estrogen system containing a human estrogen receptor (hER). These investigators showed that combinations of two weak environmental estrogens, such as dieldrin, endosulfan, or toxaphene, were 1000 times as potent in hER-mediated transactivation as any chemical alone. Because results in this paper suggested synergism among endocrine disruptors in the environment, a great deal of attention was focused on it by the scientific community, the media, and the U.S. Congress. Approximately 13 months later, the paper (8) was retracted (9) because several laboratories in the United States and Europe, including the Tulane/Xavier Center where the original work in that publication was conducted, could not repeat the studies. Although some of the scientific critics agreed that "...lots of good labs report results that can't be repeated..." and "...scientists succeed or fail in replicating studies every day..." (7), this controversy brought the issue of experimental variabilities in chemical mixture studies into focus. In our own laboratories at three different institutions, my colleagues and I had at least three such experiences of unrepeatable experiments on separate projects covering a span of many years. For instance, in a collaborative project between NIEHS and the former U.S. Environmental Protection Agency Health Effects Research Laboratory, we initially observed distinct interactive liver toxicity (serum chemistry and histopathologic changes) in Fischer 344 rats between a mixture of groundwater contaminants in drinking water and an acute dose of carbon tetrachloride. We were understandably excited about the enhancement of carbon tetrachloride liver toxicity by the preexposure of a mixture of groundwater contaminants at low, environmentally realistic levels. Because it was a one-dose preliminary study, we repeated the experiment

with more animals per group and increased the carbon tetrachloride dose levels to four doses plus control, bracketing the original dose in the initial study. In addition, we incorporated a restricted water control group because the chemical mixture caused a reduction of water intake in the animals. This time, to our surprise, we failed to observe such interactive hepatotoxicity because the reduction in water consumption caused sufficient enhancement of liver toxicity by carbon tetrachloride so as to render the toxicologic interaction between the chemical mixture and carbon tetrachloride insignificant. Our final publication (52) reported a lack of toxicologic interaction—a complete reversal from our original observation.

It is possible that this type of problem is particularly acute with chemical mixture studies, especially at low doses. Because of the multiple chemicals involved, the intrinsic animal/sample variability is amplified in the final results. Given the limitation of resources for sample size, such amplification of biologic variability may result in discrepant findings from one replicate to another.

Legislative and Regulatory Atmosphere: Driving Force for Scientific Investigations on Chemical Mixtures?

In the language of two public laws enacted recently, the specific emphasis of research work on chemical mixtures was stated. For instance, an amendment to the Clean Air Act enacted on 15 November 1990, contains such language in two sections: "...Consideration of individual, as well as complex mixtures of, air pollutants and their chemical transformations in the atmosphere..." (53) and "...In conducting the research program under this subsection, the Administrator shall develop methods and techniques necessary to identify and assess the risks to human health from both routine and accidental exposures to individual air pollutants and combinations thereof..." (54). More recently, the Safe Drinking Water Act Amendments of 1996 (55), contained the following language "...The Administrator shall conduct biomedical studies to...develop new approaches to the study of complex mixtures, such as mixtures found in drinking water, especially to determine the prospects for synergistic or antagonistic interactions that may affect the shape of the dose-response relationship of the individual chemicals and microbes, and to examine noncancer endpoints and infectious

diseases, and susceptible individuals and subpopulations... ”

Such insightful legislation, presumably under the advisement of scientists, inevitably leads to increased research funding in the area of chemical mixtures. As a researcher interested in chemical mixtures, I would hope that this type of legislation will bring about the much-needed momentum for further development of this area of research.

What Are the Pitfalls of Applying for Research Funding on Investigating Chemical Mixtures?

The application of grants using a central theme of chemical mixtures is really a catch-22. Everyone agrees that it is important to study chemical mixtures, but there is no agreement on the best way to approach such studies. In general, the more visionary, complex, unconventional, and risky a grant proposal is, the less likely it is

to be funded. The principal reason is that most reviewers get uncomfortable and nervous when a proposal is out of the norm (i.e., not what everyone else is doing). Research proposals on chemical mixtures are certainly out of the norm. Because of the intrinsic complexity associated with research on chemical mixtures, a systematic approach is usually untenable because of limitation of resources. Therein lie the potential flaws for the reviewers to uncover.

If the legislation mentioned previously (53–55) is any indication of the future trend, we should be seeing a lot of scientific activities related to chemical mixtures in the future. With the potential for an expanded research investment in the area of chemical mixtures looming on the horizon, what can we do to cultivate a workable strategy? My view is that the traditional U.S. National Institutes of Health study sections are not the answer. These peer-review bodies have served in an

outstanding manner for basic research. However, they are too specific, too molecular biology driven, and too far removed from solving the problems facing society. For research on chemical mixtures to have a chance to bear fruit, special ad hoc peer-review groups should be established. The individuals on these panels must have an appreciation of the complexity and problems related to dealing with chemical mixtures, and they must also have unusually open minds to accommodate risky research and unconventional thinking.

Conclusion

In this paper, I have attempted to express my views on a number of issues critically important to the research development of the toxicology of chemical mixtures. I tried to be provocative, not to cause anger or resentment, but to invite debate or discussion. Hopefully, through this type of exchange, we will be better thinkers and investigators.

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