Using Information on Exposure to Characterize Risks to Human Health from Concurrent Exposures to Multiple Chemicals

Het gebruik van blootstellingsgegevens om risico's te karakteriseren voor de mens bij gelijktijdige blootstelling aan meerdere chemicaliën.

(met een samenvatting in het Nederlands)

Proefschrift

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Cover piece and back piece are numbers 23 and 2 of the series of woodblock prints by Utagawa Hiroshige entitled *Thirty six Views of Mount Fuji* (circa 1852). The cover presents a seaside where the right side and foreground are dominated by the chaos of surf on a rugged coastline and two threatened boats. This is framed, on the left and in the background, by a tranquil scene of a sailing vessel, calm seas, flocks of bird, and of course Mount Fuji. This thesis proposed ideas and concepts that address the complexities and challenges of chemical mixture risk assessments and seeks to place them within an ordered context for investigation and management. The back cover presents a street scene outside of the Mitsukoshi, a 19th century department store, in the merchants' district of Edo. The exposures to complex chemical mixtures that raise regulatory concerns are those that arise from societies' technological and commercial activities.

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Definitions

One of the challenges in assessing the risks associated with exposures to mixtures is the competing and contradictory definitions are currently used to describe mixtures. The following definitions will be used in this thesis.

Aggregate exposure refers to individuals' exposures to a single compound from multiple sources.

Cumulative exposure refers to individuals' exposures to multiple compounds from multiple sources.

Exposure to mixtures refers to individuals' exposures to multiple compounds from an individual's interaction with group of chemicals in an environmental media, formulation, or food.

The following table presents the framework for the definitions.

	One chemical	**More than one chemical**
One source	*Traditional Risk Assessment*	*Mixture Risk Assessment*
More than one source	*Aggregate Risk Assessment*	*Cumulative Risk Assessment*

A concurrent exposure refers to exposures to two or more chemicals that occur at the same time or at times sufficiently close that the individual has not recovered from the effects of a prior exposures.

Chapter 1

General Introduction[1]

[1] The materials in chapters one, eight, and nine of this thesis are entirely the author's and do not necessarily reflect the positions or opinions of The Dow Chemical Company.

BACKGROUND

Findings from the fields of pharmacology, toxicology, and epidemiology have shown that cumulative exposures can cause effects that would not have been predicted based on the independent effects of chemicals [1, 2]. Studies of the interaction of the toxicity of chemicals began more than 90 years ago [3]. In the U.S. the issue of cumulative exposures of chemicals in the workplace was first raised in 1963 [4] and in the environmentally mediated exposures in 1980 [5]. Despite this long history of study, assessments of the risks posed by chemicals have traditionally evaluated chemicals on a chemical-by-chemical basis.

Assessments of the risks from concurrent exposures to multiple chemicals present major challenges to exposure assessors, toxicologists, risk assessors, and risk managers. In addition, the available approaches for assessing cumulative risks have drawbacks which limit the number of cumulative risk assessments that have been performed. In this section, we review the issue of health effects posed to individuals by concurrent exposures to multiple chemicals and identify the challenges with regard to assessing risk from these exposures[2]. As subsequent chapters indicate, at least partial solutions to a number of the challenges have been developed.

Why is there a concern with cumulative exposures?

The toxicological concerns for concurrent exposures are based on two lines of argument. The first is the recognition of the increasing dominance of synthetic materials in the everyday life of humanity resulting in continuous exposures to combinations of anthropogenic chemicals. The second is the potential for additive effects from concurrent exposures to multiple chemicals and potentially supra-additive effects (synergy[3]) for certain combinations of chemicals.

Humans are increasingly exposed to complex combinations of anthropogenic chemicals [6]. Over the last 400 years humanity has steadily moved from an environment dominated by natural chemicals to one dominated by anthropogenic chemicals. In Europe, the U.S., Japan, and other countries, an individual can go through an entire day and touch no object, come in contact with no fabric,

[2] This thesis focuses on noncarcinogenic effects in humans where thresholds are used as the basis for determining response. Mixtures are a concern for carcinogens but the existing policies that favor non-threshold approaches for this endpoint greatly simplify the issue of cumulative risks. With an assumption of no thresholds, dose addition and response addition models of cumulative exposures give the same answer.

[3] Antagonism is of course also possible. But antagonism is of little concern for public health since it reduces the effects of the cumulative exposures.

consume no food or beverage that is not, at least in part, the creation of the modern chemical industry. The ubiquity of anthropogenic chemicals has raised the question of how to protect humanity in this new environment not from any one chemical but from all chemicals. Supporting this new view are calls to study the relationship between individuals' total exposures to all stressors and existing disease patterns in a systematic way [7 - 9].

Concurrent exposure to multiple chemicals that exert the same type of a stress on individuals may produce additive effects. Examples of such an interaction include the organophosphorous pesticides [10] where multiple chemicals all interact with a common target (acetylcholinesterase). The public health concerns driven by these models suggest that while any one chemical's stress on an individual can be tolerated, the cumulative stress from multiple chemicals can exceed the capacity of an individual. In addition, when the toxicity pathways leading to an apical effect of a chemical have multiple steps, additivity could occur when different chemicals impact different steps in a toxicity pathway [11]. This type of toxicological interaction can be characterized using additive models [12, 13, 1].

Additive models are attractive to risk assessors because they can be applied in a tiered fashion. The models can begin with the conservative assumption that all chemicals that reach an individual follow an additive model. This assumption can then be refined using chemical specific data on mode of action [12, 14] to produce more realistic estimates of cumulative effects.

The second toxicological issue is the concept of synergy. Synergy is a supra-additive response from multiple exposures to chemicals. Examples of synergy have been identified in epidemiology and a modest number of animal studies [2]. The concern raised by synergy is that sub-threshold exposures to two or more chemicals could cause an adverse effect by synergy that would not be accounted for in an additive model. An additional concern is that synergy is an emergent property that cannot be predicted based on the toxicological properties of individual chemicals.

A combination of the additive and synergistic arguments has led the authors of a recent National Academies of Science report [15], to posit that the existence of background stress from chemicals in diet, nonchemical stressors, and microbial agents, create sub-populations of marginal individuals who are on the "verge of experiencing" an adverse effect. Such hypersensitive individuals will be pushed over the "threshold" of an adverse effect by even very small doses of a chemical [16, 17].
These toxicological models have combined to produce a widespread concern that regulatory toxicology programs determining the safety of chemicals on a chemical-

by-chemical basis may be missing adverse effects from cumulative chemical exposures. As a result of these arguments, there is a widespread recognition that cumulative exposures to chemicals are the unfinished business of toxicology and chemical regulation [1]. Regulatory agencies in the United States and European Union have identified effects from cumulative exposures to chemicals as area that should be addressed in future regulatory programs [17, 1].

The conundrum of cumulative exposures

Concerns for the potential for adverse effects from cumulative exposures to anthropogenic chemicals tempered, in part, by the recognition that life evolved in the presence of complex stressors. For example, the human diet has always been a complex mixture of chemicals, many with significant biological activity [18 - 20]. Thus biological systems must have an inherent capacity to tolerate multiple chemical stressors.

The increasing technological environment in industrialized nations also has been associated with remarkable improvements in public health and continuous increases in life span. This has occurred in spite of concurrent increases in populations. Thus on a societal level the benefits of technology have outweighed whatever cumulative adverse effects have accompanied the rise of modern chemistry. Such a finding, however, is not a proof that the adverse effects do not exist or that there would be no benefit to society from identifying and addressing specific chemicals, sources, and human populations that may be at risk from the effects from combined chemical exposures.

Assessing toxicity from exposures to multiple chemicals

A number of guidance documents published by the World Health Organization, United States Environmental Protection Agency (U.S. EPA), and other organizations have identified two basic approaches to characterize risks from concurrent exposures to multiple chemicals, whole-mixture testing and estimating toxicity based on the toxicity of the components of individual chemicals [12, 13, 21 - 23].

Whole-mixture testing has been limited by the fact that there are for all practical purposes an infinite number of mixtures that humans come in contact with during their lives. In addition, many mixtures change over time. For example, the specific composition of mixtures of anthropogenic chemicals in indoor air, food, and water vary from day to day. As a result, whole-mixture approaches for assessing toxicity have been limited to testing of products with fixed formulations or commercial mixtures where the composition is relatively stable over time (e.g., commercial solvents and petroleum products). Currently a number of regulatory programs

require testing of products for acute toxicity and local effects [1, 21]. Whole-mixture testing has had a more extensive use in ecological toxicity [24] where whole-mixture toxicity has been determined for complex mixtures in effluents. Whole-mixture approaches cannot be used to address cumulative exposures, since these exposures are not the result of exposure to specific mixtures.

As a result of the above challenges, many programs that assess mixtures and cumulative exposures rely on component-based approaches. For example, a hazard quotient/ hazard index (HQ/HI) process is used to evaluate occupational exposures to mixtures at hazardous waste sites in the U.S. [4, 23]. In addition, the U.S. EPA's programs to regulate cumulative risks under the 1996 Food Quality Protection Act (FQPA) have used toxicity equivalents approaches [1] to evaluate pesticides.

THE ROLE OF EXPOSURE AND TOXICITY INFORMATION IN ASSESSING AND MANAGING RISKS FROM CUMULATIVE EXPOSURES

Historical bias towards toxicity data in mixture assessments

One of the reasons why mixtures are a difficult issue for regulators is that traditionally most of the emphasis in cumulative risk assessments has focused on using the data on the toxicology of mixtures and has not considered the data on exposures. Toxicologists studying mixtures have focused on those mixtures that have interesting toxicological properties, but are not necessarily representative of the mixtures that actually occur in the real world or the combinations that individuals are exposed to from cumulative exposures. For example, one common approach is to test mixtures where each component is present at equitoxic doses (ED_{50}s, ED_{10}s, or some fraction of these values). Such mixtures facilitate the determination of whether the mixture follows additivity or independence models or if synergy has occurred. However, most mixtures, even if they are composed of large number of chemicals, are driven by the toxicity of one or two components [25, 26].

A second challenge to studies of mixtures is the practical limitation of testing. Discrimination between toxicological mechanisms can only be empirically determined when animals are dosed at levels where significant responses occur. This restricts testing to doses that cause frank effect levels. Such studies produce findings that are not directly relevant to environmental mixtures where all chemicals are kept at levels below regulatory standards. Thus the toxicological findings must be extrapolated to mixture exposures that are far below the tested doses.

The historical dominance of toxicological data in assessing mixtures can be seen in the guidance on mixtures provided by the U.S. EPA in 2000 and 2007 [12, 13]. In the following flow chart (Figure 1), the decisions on the assessment of risks from mixtures are only based on the management of the limitations of data available data and the selection of the dose response model of mixture toxicity.

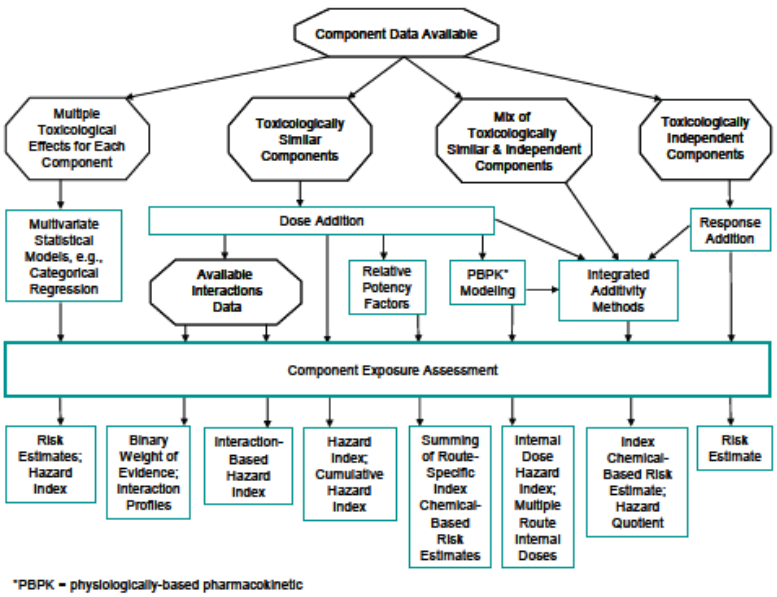

Figure 1. Decision flow chart from the U.S. EPA 2000 and 2007 guidance documents [12, 13]

The emerging recognition of the importance of exposure information in the management of cumulative exposures

This focus on toxicity has changed over time. Figure 2 presents the mixture strategy as developed by the WHO [14, 27). As the figure indicates in this tiered approach, exposure and toxicity are evaluated in parallel. Each decision to go to a higher tier requires more detailed data for both exposure and toxicity. Under this approach data on toxicity and exposure are given equal weight.

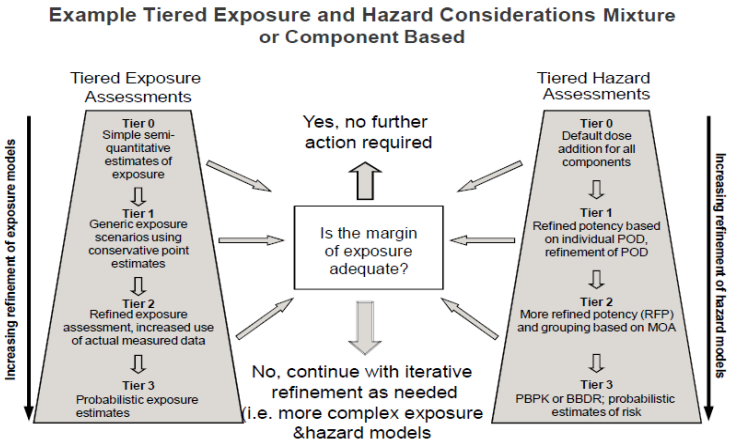

Figure 2. WHO tiered approach for mixtures [27]

More recently, the Cefic MIAT team [28] has proposed an integrated approach that integrates the collection of data on toxicity and exposure (Figure 3). This decision tree is built on the decision tree present in the draft guidance from the EU Scientific Committees [29]. Both trees begin with decisions based on exposure rather than toxicity. The focus on exposure information in these decision trees has a number of benefits. First it considers when co-occurrence of chemicals actually occurs. If the chemicals do not co-occur, then cumulative exposures do not occur and an assessment is not needed. Second, in the vast majority of cases, individuals who are exposed to many chemicals actually have their risks driven by only a small fraction of chemicals. As a result the risks from exposure to complex mixtures may be managed by controlling a subgroup of chemicals [30]. Third, some chemicals are only present in small amounts and the resulting low doses to individuals can be characterized by using what the WHO call the Tier 0 assumptions for toxicity. An example of such assumptions is the use of the Threshold of Toxicological concerns [31].

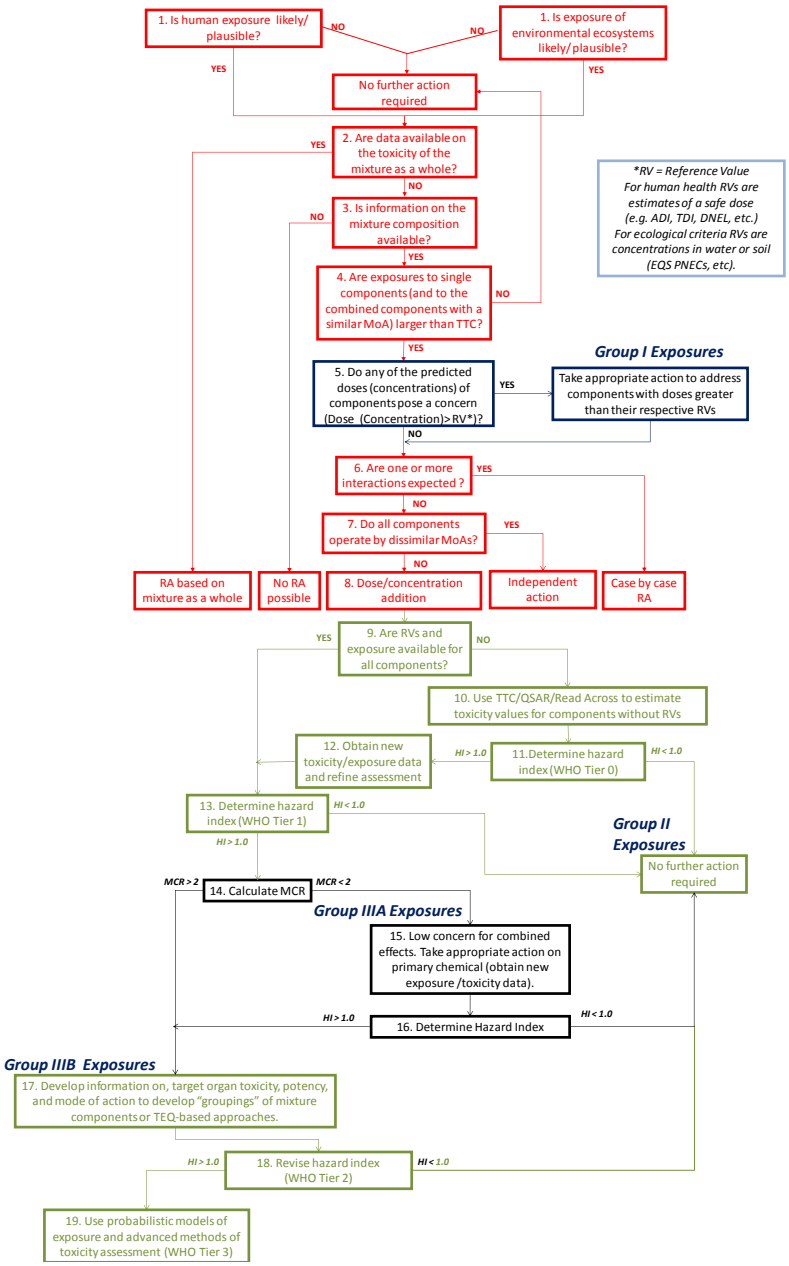

Figure 3. Cefic MIAT decision tree [28]

Characterizing Cumulative Exposures to Chemicals

Methodologies for assessing cumulative exposures to multiple chemicals from multiples sources fall into two broad classes; monitoring and modelling. Monitoring approaches collect data on the presence of sources of exposure in individuals' lives at specific points in time and the factors that determine the uptake of chemicals from these sources. Monitoring include collection of data on the levels of chemicals in food, air, water, and on the surfaces in residences and the collection of demographic and behavioural information (e.g., use of consumer products, activity patterns, employment, etc.). Monitoring also includes biomonitoring of chemicals or markers for chemicals in individuals' bodies (e.g. protein adducts). Biomonitoring data can reflect both aggregate and cumulative exposes for individuals. When the body burden or internal markers of exposures have long half lives, biomonitoring can also reflect the impacts of exposures over long periods of time [6, 32, 33]. Recent advances in personal monitoring allowing for collection of longitudinal data (information on how individual's chemical exposures change over time) and how individuals physically move through different microenviroments in their communities [34].

Monitoring data, however, cannot investigate all chemicals that reach humans. The list of chemicals included in monitoring programs is typically limited by the capabilities of analytical chemistry and the cost of analyzing for chemicals. A larger issue with monitoring is that it cannot look into the future. Thus monitoring cannot be used to assist in the evaluation of chemicals before they enter commerce.

The second method for characterizing cumulative exposures is simulation modelling. The goal of simulation models is to capture the processes that determine exposures (e.g., use of products, duration of time in micro-environments where chemicals are present, and fate and transport of chemicals) and behaviours of individuals that influence uptake of chemicals (e.g., breathing rates, frequency of hand-to-mouth events, and food and water consumption rates). This is done by collection of data on the parameters, using dose models, and construction of simulation models of individuals over time [35-36].

Models and monitoring are complementary. Models can guide monitoring efforts by identifying the aspects of a community that are most important to measure or portions of the community most at risk. Models can leverage monitoring data, predicting if, or when, monitoring results could differ in untested populations. In addition, simulation models can be used to predict future exposures. Monitoring data can provide key information on exposures that cannot be determined using physics-based models. An example of this is the use of survey data on food consumption and daily activity patterns used in the LifeLine model [38]. Finally,

monitoring data can be used to evaluate and in certain limited areas, validate model predictions [39].

ISSUES WITH MODELING RISK FROM CUMULATIVE EXPOSURES TO CHEMICALS

As discussed in the background section the current system of managing chemical risks has great difficulties in addressing risks from cumulative exposures to chemicals. The following is a list of some of the key challenges that have been identified in cumulative risk assessments and that may have discouraged the regular performance of such assessments.

High cost of determining permitted doses of chemicals

Current approaches for the evaluation of the safety of chemicals require the use of dozens of lengthy and resource intensive animal tests. For example, the U.S. EPA registration process for a single pesticide requires the manufacturer to conduct, analyze, and pay for some 142 different scientific tests. These tests define product chemistry, risks to humans and domestic animals, the environmental fate of the pesticide, and the pesticide's impact on nontarget wildlife. Generation of such data for a single chemical may take 6-10 years, cost tens of millions of dollars, and require large number of animals [40]. There is a growing consensus that such intensive levels of testing will never be performed on all of the chemicals in commerce [41].

This high cost of testing affects both whole-mixture and component-based methods of testing the toxicity of cumulative exposures. First, if the existing approach for determining safety of chemicals will not address risks from individual chemicals, then it will never be capable of directly testing all of the possible combinations of chemicals that are received by a specific person. Second, the evaluation of a mixture using a component-based approach requires information on the toxicity of every component. As a result, assessing an environmental mixture could require toxicity information on dozens or hundreds of chemicals. In theory, the absence of information on any one of these chemicals will prevent a complete determination of risks from cumulative exposure.

Existing studies of whole-mixture toxicity have largely been limited to acute effects

Toxicologists studying mixtures have been limited to studies of acute toxicity. The reason for this is that mixture study designs require testing of individual components as well as chemical mixtures. This results in study designs that use

large amounts of animals and dose groups. Such studies become prohibitively expensive if they are performed over long periods of time (months or years). As a result study designs for mixtures have focused on acute effects.

Biased nature of existing permitted doses

The design of animal testing and assumptions and processes used in setting permitted doses (ADIs, RfDs, DNELS, and TDIs) are optimized to minimize false negatives. This is reasonable and appropriate for standards. As a result, however, there is a greater than 50 percent probability that the actual population threshold of safe doses could be ten-fold higher than the proposed permitted doses for most chemicals [42]. In the case of mixtures this bias is compounded since additive models use the permitted doses for multiple chemicals. If for example, the current approach will overestimate the toxicity 90% of the time then the use of two standards in an additive model of a two equitoxic doses will overestimate toxicity 99% of the time [43].

Emergent toxicological properties cannot be predicted using component approaches

As discussed above, synergy cannot be predicted using component-based approaches. Synergy is a public health concern, since the presence of synergy could result in higher risks than predicted by additive models. Demonstration of synergy in animals has typically required testing in dose ranges that cause frank effects for one or more chemicals in a mixture. The observed responses are compared to the responses predicted by additive models. It is not clear in these studies whether synergy increases the response at unsafe doses of chemicals, or if synergy reduces the threshold to mixture effects. As a result, a critical issue in mixtures toxicity is to determine whether the threshold of interaction is always higher than the threshold of one or more components of the mixture.

The challenge of characterizing cumulative exposures

Cumulative exposures are difficult to characterize. Routes of exposure are often poorly understood or difficult to characterize on a quantitative basis. Information, the correlation of the use of products or behaviour that will define individuals cumulative exposures are often unknown [44]. Data on the behaviours of individuals are often limited and collected in ways that can generate biased data [45].

Access to monitoring data is often limited by claims of confidentiality.

Many organizations that collect data on mixtures observed in indoor air or the environment release only summary statistics. Mixture assessments require access to the raw data collected. Specifically, they require the concentrations of each

chemical measured in each sample along with data on the detection limit for the analytical method used on a sample. Obtaining access to the raw data is often limited by claims that the raw data is the property of the collecting organization.

Model validation

The evaluation of model predictions is a major challenge. The goals of modelling are often difficult to independently confirm. For example, models are often used to predict chronic exposures to chemicals from dietary exposures. However, monitoring data is often limited to information on levels of contaminants in specific food items and studies of intakes are often limited to a few days. Thus, a model's predictions of acute exposures across individuals could be evaluated by methods such as biomonitoring but the predictions of interest, chronic exposures, cannot be easily confirmed.

GOAL OF THE THESIS

This thesis explores a number of techniques that can assist in performing cumulative exposure assessments and using the resulting data in cumulative risk assessments and risk management. A general theme in these techniques is the importance of understanding the exposures to the different chemicals that make up an individual's cumulative exposures. Such information can be used to determine when screening assumptions can be used for estimating toxicity of specific chemicals and when the toxicity of mixtures becomes dominated by a few chemicals. Data on mixtures can also be used to determine which components in a mixture are most likely to interact. Such information can be used to prioritize when additional data should be collected. Finally, the information can be used to determine when cumulative assessments should be performed.

OUTLINE OF THE THESIS

This thesis presents the results of research performed on four aspects of cumulative risk assessments.

1. Using simulation models to characterize cumulative exposures to multiple chemicals from multiple sources.
2. Developing a framework to assessing the risk management implications of toxicological findings of synergy.
3. Screening approaches for assessing the toxicity of mixtures where some or all of the components do not have toxicity data.

4. Using exposure information to evaluate the need for performing cumulative risk assessments.

Focus 1. Using simulation models to characterize cumulative exposures to chemicals.

Chapter 2. Assessing Aggregate and Cumulative Pesticide Risks Using a Probabilistic Model

As discussed above, simulation models are a useful method for assessing cumulative exposures across a population. In 1994 the U.S. Congress passed the FQPA. This Act required the U.S. EPA to regulate the aggregate and cumulative risks posed by pesticides. This requirement triggered a number of private and governmental projects to develop simulation models for pesticides. One of the projects created the LifeLine™ software. When toxicity equivalents are available, this software can evaluate the variation in individuals' cumulative exposures to multiple chemicals from multiple sources. Chapter two presents the results of the application of the software to three hypothetical pesticides.

Chapter 3. A Conceptual Framework for Modelling Aggregate and Cumulative Exposures to Chemicals

Chapter 3 presents a modelling framework for determining longitudinal cumulative exposures to populations of individual humans. The framework call Person Oriented Modelling (POM) provides guidance for how to organize existing data for constructing computer software models using a series of nested loops. The methodology also provides guidance for the design of the software code. Variations of this methodology provide the basic design of many cumulative exposure models [46].

Focus 2. Developing a framework to assessing the risk management implications of toxicological findings of synergy.

Chapter 4. Synergy A Risk Management Perspective

This chapter reviews the concept of synergy and its potential for the impact on regulation of cumulative exposures or exposures to mixtures. A method for graphically characterizing responses from mixtures is provided that can help identify which components of a complex mixture are most likely to drive an observation of synergy.

Focus 3. Screening approaches for assessing the toxicity of mixture components with there is no toxicological data available on one or more components of the mixture.

Chapter 5. Characterizing the noncancer toxicity of mixtures using concepts from the TTC and quantitative models of uncertainty in mixture toxicity
Chapter 6. Modelling the chronic non-cancer effects of mixtures of migrants using Cramer classes and quantitative models of uncertainty
These two chapters present an example of a WHO Tier 0 assessment methodology [14] to mixtures of anthropogenic chemicals that migrate from food contact materials. The methodology allows a tiered approach to assessing mixtures where certain components do not have toxicity data. Toxicity data for these components are derived based on the Cramer Classes [31] of the components structures.

Focus 4. Use of exposure information to evaluate the need for performing cumulative risk assessments.

Chapter 7. Maximum Cumulative Ratio (MCR): A Tool for Assessing the Value of Performing a Cumulative Risk Assessment
This paper describes MCR, a new method for understanding the risks associated with cumulative risk assessments. This approach allows the determination of the value in performing a cumulative risk assessment. The methodology is applied to a data set of observed mixtures of pesticides in samples of surface water taken from urban and rural areas of the U.S.

Chapter 8 presents a summary of the finding from the four focus areas and discusses how the effort addresses the issues with charactering risks from cumulative exposures. Finally, chapter 9 presents a summary of the findings of the work presented in this thesis and recommendation for next steps in mixture research.

REFERENCES

1. Kortenkamp A, Backhaus T, Faust M. 2009. State of the Art Report on Mixture Toxicity Final Report, Study Contract Number 070307/2007/485103/ETU/D.1, 22 December
2. 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, Zaleski R. 2011. Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment. Crit Rev Toxicol. 2011 Feb 10.
3. Bliss C. 1939. The Toxicity of Poisons Applied Jointly. Annals of Applied Biology. 26:585-615
4. ACGIH. 1963. Transactions of the Twenty-Fifth Annual Meeting of the American Conference of Governmental Industrial Hygienists, May 6–10, Cincinnati, Ohio.
5. National Research Council. 1980. Problems of Risk Estimation Drinking Water and Health, Volume 3. Washington, DC: The National Academies Press

6. CDC, 2009. Fourth National Report on Human Exposure to Environmental Chemicals, Department of Health and Human Services, Centers for Disease Control and Prevention Down loaded 15 January 2012. http://www.cdc.gov/exposurereport/pdf/FourthReport.pdf
7. Wild CP. 2005. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev 14(8):1847–1850.
8. Rappaport S. 2011. Implications of the exposome for exposure science Journal of Exposure Science and Environmental Epidemiology. 21, 5–9; doi:10.1038/jes.2010.50; published online 17 November 2010
9. Smith M, Rappaport S. 2009. Building Exposure Biology Centers to Put the E into "G × E" Interaction Studies Environmental Health Perspectives, Vol 117 (8) 334-335. doi:10.1289/ehp.12812
10. Bosgra S, van der Voet H, Boon P, Slob W. 2009. An integrated probabilistic framework for cumulative risk assessment of common mechanism chemicals in food: An example with organophosphorus pesticides. Reg Tox and Pharm, Vol 54 (2) Pg. 124-133 doi:10.1016/j.yrtph. 2009.03.004
11. National Research Council. 2008. Phthalates and Cumulative Risk Assessment, Washington, DC: The National Academies Press
12. U.S. EPA. 2000. Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum, Office of Research and Development, Washington, DC, EPA 630/R-00-002
13. U.S. EPA. 2007. Concepts, methods and data sources for cumulative health risk assessment of multiple chemicals, exposures and effects: A resource document. Cincinnati, OH: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, [EPA/600/R-06/013F].
14. WHO. 2009. Harmonization Project Document 7; Assessment of Combined Exposures to Multiple Chemicals: Report of a WHO/IPCS International Workshop on Aggregate/Cumulative Risk Assessment; WHO: Geneva, Switzerland
15. National Research Council. 2008. Science and Decisions: Advancing Risk Assessment. Committee on Improving Risk Analysis Approaches Used by the U.S. EPA. National Research Council, Washington, DC.
16. Hattis D, Baird S, Goble R. 2002. A STRAW MAN PROPOSAL FOR A QUANTITATIVE DEFINITION OF THE RfD, Drug Chemical Toxicology Vol. 25, No. 4 , Pages 403-436 (doi:10.1081/DCT-120014793)
17. Goldman, L. 2009. Toxic Chemicals and Pesticides: Not Yet Preventing Pollution. In Agenda for a Sustainable America (Environmental Law Institute), Ed. John Dernbach Washington DC ISBN 978-1-58576-133-3
18. Hirono I, Grasso P. 1981. Natural Carcinogenic Products of Plant Origin, Critical Reviews in Toxicology, Vol. 8, No. 3 , Pages 235-277 (doi:10.3109/10408448109109659)
19. Carney E, Woodburn, K, and Rowland J. 2010. Endocrine active chemicals. In Principles and Practice of Mixtures Toxicology (M. M. Mumtaz, Ed.), pp. 421–442. WILEY-VCH Verlag GmbH & Co., Weinheim, Germany.
20. Barceloux D. 2008. Medical toxicology of natural substances: foods, fungi, medicinal herbs, plants, and venomous animals. John Wiley and Sons, Nov 12, 2008 - Medical - 1157 pages
21. Interdepartmental Group on Health Risks from Chemicals (IGHRC). 2007. Chemical mixtures: A framework for assessing risks. http://ieh.cranfield.ac.uk/IGHRC/mixtures document.pdf.
22. Agency for Toxic Substances and Disease Registry (ATSDR). 2004. Guidance Manual for the Assessment of Joint Toxic Actions of Chemical Mixtures. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service
23. U.S. EPA. 1989. Risk Assessment Guidance for Superfund Volume I Human Health Evaluation Manual (Part A) Interim Final. Office of Emergency and Remedial Response. EPA/540/1-89/002

24. Brack W. 2003. Effect-directed analysis: a promising tool for the identification of organic toxicants in complex mixtures? Analytical and Bioanalytical Chemistry Volume 377, Number 3, 397-407, DOI: 10.1007/s00216-003-2139-z
25. Han X, Price P. 2011. Determining the Maximum Cumulative Ratios for Mixtures Observed in Ground Water Wells Used as Drinking Water Supplies in the United State Int. J. Environ. Res. Public Health 2011, 8(12), 4729-4745; doi:10.3390/ijerph8124729 (http://www.mdpi.com/1660-4601/8/12/4729/)
26. Junghans, M.; Backhaus, T.; Faust, M.; Scholze, T.; Grimme, LH. Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. Aquat. Toxicol. 2006, 76, 93-110.
27. Meek M, Boobis A, Crofton K, Heinemeyer G, Van Raaij M, Vickers C. 2011. Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. Regul. Toxicol. Pharmacol. Vol. 60, (S1-S14)
28. Cefic. 2011. Comments provided to on the Scientific Committees report Toxicity and Assessment of Chemical Mixtures, 16 September
29. Scientific Committees (Scientific Committee on Consumer Safety (SCCS) Scientific Committee on Health and Environmental Risks (SCHER) Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). 2011. Toxicity and Assessment of Chemical Mixtures (Preliminary Opinion approved for Public Consultation), European Commission, DG Health & Consumers, June 21
30. Kortenkamp A, and Faust M. 2010. Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment. Internat J Androl 33, 463
31. Munro I, Ford R, Kennepohl E, Sprenger J. Correlation of structural class with no-observed-effect levels: A proposal for establishing a threshold of concern. Food and Chemical Toxicology, 1996; 34(9):829–867.
32. Rubino F, Pitton M, Di Fabio D, Colomb A. 2009. Toward an ''Omic'' Physiopathology of Reactive
33. Chemicals: Thirty Years of Mass Spectrometric Study of the Protein Adducts with Endogenous and Xenobiotic Compounds, Mass Spectrometry Reviews, 28(5):725-84
34. Calafat AM. 2011. The U.S. National Health and Nutrition Examination Survey and human exposure to environmental chemicals. Int J Hyg Environ Health. Sep 19. [Epub ahead of print]
35. Borrell, B. 2011. Every Bite You Take. *Nature* **470**, 319-321.
36. Price P, Chaisson C. 2005. A conceptual framework for modelling aggregate and cumulative exposures to chemicals. *Journal of Exposure Analysis and Environmental Epidemiology* **15**, 473-481, DOI 10.1038/sj.jea.7500425.
37. Price P, Young J, and Chaisson F. 2001. Assessing aggregate and cumulative pesticide risks using a probabilistic model. Annals of Occupational Hygiene 45, S131-S142
38. Georgopoulos P, Wang S, Yang Y, Xue J, Zartarian V, McCurdy T, and Ozkaynak K. 2008. Biologically based modelling of multimedia, multipathway, multiroute population exposures to arsenic. *Journal* of Exposure Science and Environmental Epidemiology 18, 462-476.
39. Lifeline, Technical manual LifeLine™ Version 4.3 Software. 2006, the Lifeline Group Inc.
40. http://www.thelifelinegroup.org/lifeline/docs.php
41. WHO. 2005. Principles of characterizing and applying human exposure models, Harmonization Project Document No. 3, World Health Organization, International Labour Organization and the United Nations Environment Programme, and The Inter-Organization Programme for the Sound Management of Chemicals. ISBN 92 4 156311 7.
42. Whitford F. 2001. Perdue Pesticide Program Pesticides and Wildlife: An Introduction to Testing, Registration, and Risk Management http://www.ppp.purdue.edu/Pubs/PPP30.html Accessed 2 January 2012
43. National Research Council. 2007. Toxicity Testing in the 21st Century A Vision and A Strategy. Committee on Toxicity Testing and Assessment. National Research Council, Washington, DC.
44. Gaylor, D.W. and Kodell, R.L. (2000) Percentiles of the product of uncertainty factors for establishing probabilistic reference doses. Risk Anal., 20, 245–250.

45. Carlson-Lynch H, Price PS, Swartout JC, Dourson ML, Keenan RE. 1999. Application of Quantitative information on the uncertainty in the RfD to noncarcinogenic risk assessments. Human and Ecological Risk Assessment, 5(3):527–546.
46. Leclercq C, Arcella D, Le Donne C, Piccinelli R, Sette S, and Soggiu M. 2003. Stochastic modelling of human exposure to food chemicals and nutrients within the "Monte Carlo" project: An exploration of the influence of brand loyalty and market share on intake estimates of intense sweeteners from sugar-free soft drinks, Toxicology Letters, Volumes 140–141, 11 April 2003, Pages 443–457
47. Price, P, Su S., and Gray M. 1994. The effect of sampling bias on estimates of angler consumption rates in creel surveys. J. Exp. Anal. Environ. Epid. 4(2):355-372
48. Price P, Chaisson C, Koontz M, Ryan B, Wilkes C, Macintosh D, Georgopoulos P. 2003. Construction of a comprehensive chemical exposure framework using person-oriented modelling. The Lifeline Group. Developed for The Exposure Technical Implementation Panel American Chemistry Council Contract. # 1388. Available online at
49. http://www.thelifelinegroup.org/lifeline/documents/comprehensive_chemical_exposure_framework.pdf.

Chapter 2

Assessing aggregate and cumulative pesticide risks using a probabilistic model

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Abstract

Determining aggregate and cumulative risks from exposures to pesticides presents a number of challenges. The analysis must capture the correlations in residues that occur from both additive and exclusionary processes in the use of pesticides. The analysis also requires a quantitative mechanism for evaluating risks associated with exposures to mixtures of pesticides. This paper presents an analysis of aggregate exposures and risks associated with exposures to a pesticide, Alpha, and the cumulative exposure to and risk from three pesticides, Alpha, Beta, and Gamma. The cumulative risks are evaluated by determining the systemic (absorbed) doses that result from inhalation, dermal, and oral exposures to the pesticides. A 'relative toxicity' model is used to evaluate cumulative risks. The assessment of cumulative exposure was performed using the LifeLine™ Version 1.0. The model simulates pesticide exposure using an individual-based approach where daily exposures are evaluated for each person, season, and location.

Keywords: pesticide; exposure; risk; aggregate; cumulative; probabilistic

Introduction

Historically, exposure assessors have focused on characterizing the highest levels of exposure that will occur to an individual or a population over time as the result of the use of a pesticide. One approach that is used to characterize the upper bound of exposure is to use simple models of dose rates and a series of conservative model inputs. This approach has great value for screening out exposures that are of little concern (US EPA, 1992a). This approach forms the basis for US EPA exposure guidance such as the draft Standard Operating Procedures (SOPs) for Residential Exposure Assessments (US EPA, 1998).

The difficulty with applying these approaches to aggregate or cumulative exposures is that an individual who receives high levels of exposure from one source will not necessarily receive high levels of exposure from a second or a third source.

An alternate approach is to simulate the doses received from multiple sources by individuals in a population. Monte Carlo analysis is often used for these simulations (McKone and Ryan, 1989; McKone and Daniels, 1991). Such simulations can allow the incorporation of data from multiple surveys. In addition,

Monte Carlo analysis is equally applicable for simple or complex exposure models (Morgan and Henrion, 1990). This allows for the simulation of multiple sources of exposure and multiple pesticides. Finally, the technique can be applied to complex time-dependent exposure models that follow individuals through multiple microenvironments (Price *et al*., 1992, 1996; Keenan *et al*., 1993; Harrington *et al*., 1995; Goodrum *et al*., 1996; Muir *et al*., 1998).

This paper presents an assessment of aggregate pesticide exposures (multiple sources of one pesticide) and cumulative pesticide exposures (multiple sources of multiple pesticides), and their attendant risks, using such a probabilistic model. The model used was LifeLine™ Version 1.0. This model was developed as part of a project to create and widely distribute transparent and consistent modelling tools for characterizing pesticide exposure (Price *et al*., 1999). The project has received funding from US EPA and USDA under co-operative agreements and has received additional support from private industry. Historically, probabilistic modelling has been used for the evaluation of dietary and residential pesticide exposures in products such as Calendex™ (Shurdut *et al*., 1998); however, the model used in this paper is the first attempt to model longitudinal exposures to the same individual over periods longer than one season.

Model Description

The model used in this paper draws on data from a number of different surveys of exposure-related factors performed in the United States. Information on daily activity and dietary patterns is used to evaluate daily exposures for an individual. These data include:

- Natality data (Birth records), National Center for Health Statistics;
- Residential patterns. Current Population Statistics, US Census;
- The Third National Health and Nutrition Examination Survey, National Center for Health Statistics;
- American Housing Survey, US Census and Department of Housing and Urban Development;
- Nation Home and Garden Pesticide Use Survey (NHGPUS), US EPA (1992b);
- National Human Activity Pattern Survey, US EPA (1996);
- The Continuing Survey of Food Intake by Individuals (CSFII), US Department of Agriculture;
- Residential Exposure SOPs (US EPA, 1998); and
- Exposure Factors Handbook (Anon, 1997).

In addition, the model uses the following types of user-supplied information:

- Data on annual or seasonal levels of pesticide residues in agricultural commodities and specific food forms of those commodities (e.g., cooked-canned vs. raw);
- Data on the reduction or increase of residues due to food processing;
- Annual or seasonal data on the fraction of crops that might have been treated with the pesticide;
- The residential uses of the pesticide;
- Physical and chemical properties of the pesticide;
- Frequency and levels of occurrences in ground and surface drinking water supplies;
- Oral inhalation, and dermal absorption; and
- Toxicity information (NOAEL, uncertainty factors, Food Quality Protection Act (FQPA) factor, and modifying factors).

Using these data the model determines the individual's exposures by modelling where people are born, how individuals grow and age, how they move from home to home and region to region of the US, how they use or do not use pesticides, and their daily activity and dietary patterns. Using pesticide-specific information on the fraction of the dermal, oral, and inhalation exposures that are absorbed, the model calculates the total absorbed dose received from the oral, dermal, and inhalation routes for each day of the individual's life. These estimates of absorbed dose can be summed to give the total systemic (aggregate) dose that can provide the basis for assessing aggregate risk. By repeating this process many hundreds or thousands of times the model builds up a simulation of the distribution of exposures in the general population. Since the data sets in the model are taken from the general population of the United States the result is a model of variation in exposures and dose for the population of the United States.

Modelling exposures to dietary residues

The components of the model's dietary analysis modules parallel the components of the basic equation used to estimate dietary exposure and the resulting oral dose:

$$\text{Dietary Exposure} = \sum_{i} \text{Food Item}_i \times \text{Residue level}_i$$

$$\text{Dietary Dose} = \text{Dietary Exposure} \times \text{Oral Absorption}$$

where:

- Dietary exposure is the mass of the pesticide ingested over a period of time from the diet,
- Food $Item_i$ is the mass of the *i*th food item consumed on a given day,
- Residue $Level_i$ is the level of the residue in *i*th food item, and Oral Absorption[4] is the fraction of the amount of pesticide that is ingested that is absorbed into the blood stream.

The amount of each food item that an individual consumes is taken from the US Department of Agriculture's 1989–91 CSFII (1991).

The Residue $Level_i$ is taken from a distribution of residues for each food item. This distribution is generated by the model based on distributions of residues in the specific Food Forms of the raw agricultural commodities RACs[5]), the amount of each RAC/Food Form in the food item, and processes that were performed on the RAC (storage, drying, cooking, etc.) during the preparation of the food item.

The amount of each RAG/Food Form combination in a food item reported in the dietary survey is captured in a set of Recipe Files for each food reported eaten in that survey. The Recipe Files in the mode1 originate from TAS, Inc., and was made public through US EPA. These files are currently used by US EPA when evaluating the 1989–91 USDA CSFII consumption records.

Since the data from the USDA survey and the recipes tiles are contained in the model, the user only provides information on the distribution of residues found on treated commodities and/or Food Forms, the percent of each that is treated, and the effects of different types of food processing on the level of residues.

Modelling residential exposures

Estimates of exposure from residential uses of a pesticide are based on data on pest pressures collected in the NHGPUS (US EPA, 1992b). This survey determined

[4] The value of the oral absorption factor can be influenced by factors such as pesticides affinity for different foods, the pH of the gut, and the volume of food consumed in a meal. Because of the uncertainly in the actual value for this factor a value of 1.0 is typically used in dietary assessments.

[5] The term RAC (for Raw Agricultural Commodity) is used as a category descriptor for all Food Forms of that commodity, whether or not they are raw. Among the common food forms for which separate residue data might be available are Raw, Cooked, Frozen-Raw, Frozen-Cooked, Canned-Cooked, and so forth.

the frequency with which specific pests required treatment in different residential microenvironments. These data are used, along with user-supplied information on the probability that a product containing the pesticide will be used to treat specific pests in the individual's residence, to determine the probability and frequency of using each pesticide in the residence. User-supplied data on pesticide product's characteristics are then used to predict the residues on surfaces and in the air of the residences that result from the use of the pesticide.

The model also contains information on the US housing stock, including information on room sizes, air exchange rates and other factors. Using these data and the exposure equations described in US EPA guidance for residential exposure assessments (US EPA, 1998) the model estimates the exposures that occur by the oral, dermal, and inhalation routes. These data are used to estimate the absorbed doses for each route and the aggregate dose. These exposures include both the application-related exposure and the post-application exposures. The post-application exposures considered by the model include exposures that happen on the day of application and on subsequent days. Table 1 presents the equations used to determine the exposures and doses that occur by the various routes.

Modelling tapwater related exposures

Pesticide residues occur in certain water supplies primarily as the result of agricultural uses of pesticides. When a pesticide occurs in a residence's tapwater, individuals living in those residences will be exposed. The level of exposure will be a function of the level of residues in the water supply. In order to capture the variation in these levels, the model allows the input of the distributions of residues that are expected to occur in different types of water supplies. The user can input separate distributions for each of the: four Census regions; urban or rural settings; private wells, public water supplies, or 'other water supplies', and each of the four seasons.

Based on mobility data collected by the US government and data on the sources of water for different types of housing stock, the model tracks the location of the individual's residence in terms of Census region, setting, and source of water. Once the source of the water and the location of the home are determined, a seasonal residue level is assigned to the tapwater of the residence based on the appropriate season- specific distribution.

Once the levels of residues are determined, the model uses typical tapwater consumption rates to determine the oral exposure from tapwater sources. The

doses associated with the exposures are determined using information on the fraction of the pesticide that is absorbed from the gastro-intestinal tract.

Modelling Aggregate and Cumulative Risks

As discussed above, the model determines the route-specific exposures received by a specific individual at a specific time and place. These estimates of exposure are used to determine the absorbed dose that results from each route of exposure. These route-specific doses are summed to give the total systemic (or aggregate) dose. This dose is then used to evaluate aggregate risks using measures of systemic toxicity. Route-specific effects, such as dermal irritation, are not considered in these estimates of aggregate risk and should be evaluated separately.

Assessments of cumulative risk involve the construction of models of response associated with exposures to multiple pesticides. While this issue remains an area of ongoing research, one approach, toxicity equivalents, has been widely discussed. The 'toxicity equivalents' approach normalizes exposures to a series of pesticides in terms of equivalent exposure to one 'standard' or 'index' pesticide (ILSI, 1999).

Under this approach, the pesticides being modelled are assumed to have additive effects and the effect of each pesticide can be defined in terms of toxicologically equivalent dose (TEQs) of a single index pesticide. The TEQ of a compound's dose is determined by multiplying the dose times a relative toxicity factor (RTF). The RTF is based on measures of the relative toxicity of the compound and the index pesticide.

Once all of the compounds' doses are converted to TEQs, they are summed to produce a cumulative dose of the index pesticide. The cumulative risk is determined based on the cumulative dose of the index pesticide and the measures of toxicity for the index pesticide (such as no adverse effect levels (NOAELs), reference doses (RfD), or Allowable Daily Intakes (ADIs)).

When the pesticides have similar route-specific absorptions, it possible to simplify the assessment by applying the RTF, not to the exposures, but to the measure of the residue levels of the individual pesticides. Once converted to the equivalent levels of the index pesticide, the mixture of residues is viewed as a single concentration of the index pesticide. This approach has been used in this assessment.

Table 1. Equations used to evaluate daily residential exposures and dose

$$\text{Inhalation Exposure} = \sum_i Air\_Concentration_i * Inhalation\_Rate_i * Duration_i$$

$$\text{Inhalation Dose} = Inhalation\_Exposure \times Lung\_Clearance$$

$$\text{Dermal Exposure} = \sum_i Dislodg\_Res_i * Dermal\_Transfer\_Factor_i * Duration_i$$

$$\text{Dermal Dose} = Dermal\_Exposure \times Fraction\_Absorbed$$

$$\text{Oral Exposure} = Incidential\_Oral\_Exposure + Soil\_Ingestion + Grass\_Ingestion$$

$$\text{Oral Dose} = Oral\_Exposure \times GI\_Absorption$$

$$\text{Incidental Oral Exposure} = \sum_i Dislodg\_Res_i * Events_i * Hand\_Fract * Refresh_i * Saliva\_Extraction$$

$$\text{Soil Ingestion} = Soil\_Ingestion\_Rate * Soil\_Residue * Duration$$

$$\text{Grass Ingestion} = Grass\_Ingestion\_Rate * Grass\_Residue * Duration$$

Where:

$Air\_Concentration_i$:	Average concentration of a pestiside in the air of the *i*th microenvironment.
$Inhalation\_Rate_i$:	Inhalation rate of the Modeled individual in the *i*th microenvironment.
$Duration_i$:	Length of time spent in the *i*th microenvironment.
Lung_Clearance:	Fraction of the inhaled pesticide that is absorbed and is added to the individual's systemic dose.
$Dislodg\_Res_i$:	Concentration of the pestcide per unit area that can be removed by dermal contact.
$Dermal\_Transfer\_Factor_i$:	Rate of transfer of pesticide from surfaces to the individual's skin in the in the *i*th microenvironment.
Fraction_Absorbed:	Fraction of the pesticide on the skin that is absorbed and is added to the individual's systemic dose.
Incidental_Oral_Exposure:	The ingestion of pesticide that occurs when a portion of the hand is placed in the mouth.
Soil Ingestion:	The rate that an individual ingests soil.
Grass Ingestion:	The rate that an individual ingests grass.
GI_Absorption:	Fraction of the ingested pesticide that is absorbed and is added to the individual's systemic dose.
$Events_i$:	Frequency that an individual places a portion of their hand in their mouth in the *i*th microenvironment.
$Hand\_Fract_i$:	Fraction of the individual's hand placed in the mouth when in the *i*th microenvironment.
$Refresh_i$:	Ratio of the average amount of residue on the hand to dislodgable residue (refreshment fraction) when in the *i*th microenvironment.
Saliva Extraction:	Fraction of the pesticide on the hand that is extracted by saliva.
Soil Ingestion:	Amount of soil ingested per unit time.
Soil Residue:	Amount of pesticide in soil.
Grass Ingestion:	Amount of grass ingested per unit time.
Grass Residue:	Amount of pesticide in grass.

Case Studies of Aggregate and Cumulative Exposure

Characterizing exposures

This paper includes two applications of the model. The first is a characterization of the aggregate risk associated with one-day exposure to a single pesticide, Alpha.

The second application is the characterization of the aggregate risks from two pesticides Beta and Gamma and the cumulative risks from the concurrent daily exposure to three pesticides, Alpha, Beta, and Gamma[6].
Cumulative exposures are determined using an approach similar to that used to assess aggregate exposures. In the case of dietary exposures, data on the co-occurrence of the residues of the pesticides in a food are converted to TEQs for an index pesticide (in this case Alpha) and summed to give a distribution of TEQs for that food. These distributions of TEQs can be entered into the model as if they were the concentrations of a single compound.

A similar approach is used for the evaluation of tapwater exposures. Data on concurrent levels of pesticides measured in surveys of water supplies are converted to a single distribution of TEQ and entered into the model.

A somewhat different approach is used in the assessment of residential sources of exposures. For these sources of exposure, the amounts of each of the three pesticides applied during the use of a specific product are converted to the corresponding TEQs. Then the model is run with all of the products that contain any of the three compounds.

Characterizing aggregate and cumulative risks

One of the commonly used risk metrics for the evaluation of noncarcinogenic risk is the percent reference dose (%RfD). The %RfD for risks from aggregate exposures to Alpha is given by:

$$\%\mathrm{RFD}_{Alpha} = \frac{\mathrm{Aggregate\ Dose}_{alpha}}{\mathrm{PAD}_{Alpha}}$$

where:

$\mathrm{PAD}_{\mathrm{Alpha}}$ is the population-adjusted dose of Alpha. For children and women of child bearing age the PAD is defined as the reference dose (RfD) for Alpha divided by the FQPA factor. For all other ages, it is equal to the RfD. $\mathrm{AggregateDose}_{\mathrm{Alpha}}$ is the aggregate dose of Alpha produced by the model.

[6] The data used in this assessment are similar to the properties, exposures, and uses of a number of pesticides currently in use. However, the compounds are not meant to represent any specific pesticide.

Using the RTP model the cumulative risk for three pesticides, Alpha, Beta, and Gamma, would be calculated using the following equation:

$$\%\mathrm{RFD}_{Cumulative} = \frac{\mathrm{Aggregete\ Dose}_{AlphaTEQs}}{\mathrm{PAD}_{Alpha}}$$

Where:
Aggregate $\mathrm{Dose}_{\mathrm{AlphaTEQs}}$, is the aggregate dose in units of mg Alpha TEQs/kg that occurs as a result of exposures to Alpha, Beta, and Gamma.

Model inputs

Alpha is used in products that control pests on apples[7]. Table 2 presents the residue data for Alpha on apples that were collected in a market basket survey. Alpha is also used in two residential pesticide products. The first, Alpha-pump, is a pump spray applied as a crack and crevice treatment in homes. The second, Alpha-gran, is a granular product that is applied to turf using a drop spreader. Table 3 summarizes the data for these two products. Beta is used on apples and on wheat. Table 2 gives information on the concurrent levels of Beta found in the same market basket survey as Alpha. The data on the concurrent levels have been used to estimate the cumulate level of the pesticides in units of TEQ. Table 2 also presents the estimates of TEQs for the mixtures of the two pesticides. The levels in wheat were taken from field trials. Beta is used on all wheat (percent crop treated equal 100%). Gamma is used in one residential product, Gamma spray, Table 3 gives the data on this product. The amount of Gamma applied and the air levels are expressed in terms of mass of TEQs.

The agricultural uses of Alpha contaminate surface waters used for drinking water supplies in areas of the southern portion of the United States. This contamination largely occurs during spring application of the products. Levels are below the detection limit during the other seasons. Table 4 presents the cumulative distribution of Alpha residue levels for the population living in this region and using surface water supplies in the spring. The levels during the other seasons are assumed to be equal to one half of the detection limit (0.005 μg/l.).

Table 5 presents the information on the acute non-carcinogenic effects of Alpha and the toxicological factors established for the compound. Beta and Gamma have

[7] For simplicity sake we do not address the issues of food processing factors or bioaccumulation of pesticides in beef and dairy products.

been determined to cause adverse effects by mechanisms similar to that of Alpha. Using the NOAELs in Table 5, a RTF of 0.5 has been determined for Beta and a RTF of 2.0 has been determined for Gamma.

Alpha has been found to be well absorbed by the oral and inhalation routes and partially absorbed by the dermal route, the values for dermal absorption, lung clearance, and GI absorption are 0.03, 1.0, and 1.0. Beta and Gamma have absorption characteristics similar to those of Alpha.

Model Operation

The model was run with the data on Alpha and with the data on all three compounds. The model runs were performed on a PC with a Pentium III 700 MHz using Windows 2000. The model runs simulated exposure histories for 2000 individuals. Different dietary and activity patterns records were selected for each day of each season (Price *et al*., 1999).

Results

The model generates exposure histories for each of the 2000 individuals. These exposure histories consist of route- and source- specific dose estimates for each day in the lives of the 2000 individuals. These exposure histories can be mined for information on the intra-individual variation of dose and risk (the variation of dose in one individual's life by age and season) and intra-individual variation in dose and risk (variation in dose across individuals at a specific age and season.)

Because of the importance of age and season it is useful to look at the average and the maximum doses an individual receives on any day of a given season and year of his or her life. The model determines the mean exposure for each age and season and the maximum dose that occurs on any day in the season. The average seasonal dose is very useful in comparing the doses to the typical individual in the modelled population. This measure has been used to investigate how the typical aggregate and cumulative doses vary by age and season. The second measurement is the maximum dose that occurs on any day of a season. The maximum dose is useful in determining the upper bounds of the distribution of doses that occur to individuals. The figures presented in this paper are based on either the mean daily dose for a season or the highest daily dose seen on any given day during a season.

Table 2. Measurements of concentration of alpha and beta in samples take in a market basket survey

Samples	Apples			Wheat	
	Alpha (ppm)	Beta (ppm)	Cumulative (ppm TEQs)	Beta (ppm)	Cumulative (ppm TEQs)
1	0.008	0.010	0.013	0.005	0.0025
2	0.005	0.005	0.008	0.005	0.0025
3	0.005	0.019	0.015	0.005	0.0025
4	0.005	0.420	0.215	0.005	0.0025
5	0.027	0.005	0.030	0.005	0.0025
6	0.021	0.083	0.062	0.005	0.0025
7	0.005	0.007	0.008	0.005	0.0025
8	0.005	0.005	0.008	0.005	0.0025
9	0.005	0.005	0.008	0.010	0.005
10	0.005	0.363	0.186	0.014	0.007
11	0.028	0.005	0.030	0.091	0.046
12	0.005	0.005	0.008	0.005	0.0025
13	0.005	0.043	0.026	0.005	0.0025
14	0.005	0.005	0.008	0.005	0.0025
15	0.005	0.006	0.008	0.005	0.0025
16	0.005	0.078	0.044	3.990	1.995
17	0.005	0.005	0.008	0.005	0.0025
18	0.005	0.005	0.008	0.791	0.396
19	0.059	0.005	0.062	0.041	0.021
20	0.005	0.005	0.008	0.005	0.0025
21	0.005	0.005	0.008	0.005	0.0025
22	0.005	0.005	0.008	0.005	0.0025
23	0.005	0.005	0.008	0.005	0.0025
24	0.005	0.005	0.008	0.005	0.0025

Table 3. Data on residential use of Alpha and Gamma

Product	Alpha-pump	Alpha-gran	Gamma-spary
Application method	Trigger sprayer/crack and crevice	Drop-spreader/granular	Aerosol/broadcast
Location of use	Indoors	Yard (turf)	Indoors
Application rate of product	1500 mg/m	30 000 mg/m^2	1000 mg/m^2
Concentration of AI in product (as applied)	0.5%	0.5%	0.8% (by weight) 1.6% (TEQs)
Peak air concentration	0.01 mg/m^3	–[a]	0.01 mg/m^3 (by Weight) 0.02 mg/m^3 (TEQs)
Percent daily dissipation on hard surfaces	20%	–	15%
Percent daily dissipation on carpeted surfaces	10%	–	8%
Percent daily decline in dislodgable mass from turf	–	20%	–
Percent daily dissipation on turf	–	10%	–
Percent daily dissipation in soil	–	10%	–
Fraction dislodgable on hard surfaces	0.10	–	0.010
Fraction dislodgable on soft surfaces	0.02	–	0.02
Fraction dislodgable on turf	–	0.05	–
Dermal unit exposure ng/lb AI	2 400 000	6300	1 200 000
Inhalation unit exposure µg/lb AI	220 000	2900	500 000
Pests controlled	Ants, roaches, fleas, spiders	Soil dwelling insects	Ants, fleas, spiders
Market share (for all pests)	50%	30%	20%
Minimum repeat time	7 days	60 days	14 days

[a]Not applicable

Table 4. Cumulative distributions for measured levels of alpha in surface water supplies in the southern region of the US during spring

Percentile	Concentration of Alpha (μg/l.)
0	0.0
0.70	0.00
0.75	0.005
0.85	0.05
0.97	0.1
0.985	0.5
0.9925	1.0
0.997	1.25
1.0	1.5

Results of aggregate assessment

Figure 1 presents the results from each source of pesticide exposure (diet, residential, and tapwater) and the aggregate (or total) daily dose for each year and season of the 2000 individuals. The dose presented is the mean of each individual's mean daily dose over each season and age. This figure provides insight into the age and seasonal patterns of exposure and the relative contribution of each route to the total dose for the typical individual. The measurements are in units of mg/kg/day of absorbed dose.

Figures 2 and 3 present the distributions of the interindividual variation in the population's aggregate doses at two combinations of age/season, 3 year olds in winter and 35 year olds in winter. These two combinations were selected since winter exposures appear to be higher than other seasons and since children and adults appear to have different potentials for exposures. The model provides similar data (not shown) for all other combinations of age and season.

In these figures, the doses for each individual appear on the same vertical line. The doses presented are the highest daily dose seen by each individual on any day during the season from tapwater, residential use, and dietary sources and the aggregate dose from all sources. These data have also been ranked from the individual with the lowest to the highest aggregate dose. The aggregate doses of each individual are presented as gray squares. Because of the number of individuals, these gray squares appear a gray band. The relative importance of a source can be seen by the vertical distance between a symbol of a route specific dose and the band of gray squares (the aggregate doses). Routes that are the dominant contribution to an individual's aggregate dose will have a symbol that appears close to or directly on top of a gray square. Routes that make minimal contributions appear some distance below the gray squares.

Figure 4 presents the %RfD associated the aggregate doses of simulated population when the individuals for the same ages/seasons. This figure indicates that approximately 10% of three year olds had %RfDs in excess of 100 for at least one day during that season while none of the 35 years old had %RfDs in excess of 100 on any of the days.

Results of cumulative assessment

Figure 5 presents a cross section of the population's cumulative doses of Alpha, Beta, and Gamma during winter at age 3. The doses presented are the distribution of the highest daily total dose seen by each individual on any day during the season. The figure also presents the aggregate doses of the individual pesticides. All four distributions have been independently ranked by size.

Figure 6 presents the %RfD associated the aggregate dose of Alpha and the cumulative doses of Alpha, Beta, and Gamma for three year olds in winter. This figure indicates that approximately 10% of three year olds had %RfDs in excess of 100 from exposure to Alpha for at least one day per season and the fraction with %RfDs increased to 16% when the cumulative risks were determined.

Table 5. Toxicity data for Alpha, Beta, and Gamma

	Oral	Dermal	Inhalation	Systemic
Short-term NOAEL				
Alpha	5 (mg/kg-d)	5 (mg/kg-d)	5 (mg/kg-d)	5 (mg/kg-d)
Beta	10 (mg/kg-d)	10 (mg/kg-d)	10 (mg/kg-d)	10 (mg/kg-d)
Gamma	2.5 (mg/kg-d)	2.5 (mg/kg-d)	2.5 (mg/kg-d)	2.5 (mg/kg-d)
Uncertainty factor (all compounds)	100	100	100	100
Modifying factor (all compounds)	1	1	1	1
PQPA factor (all compounds)	10	10	10	10

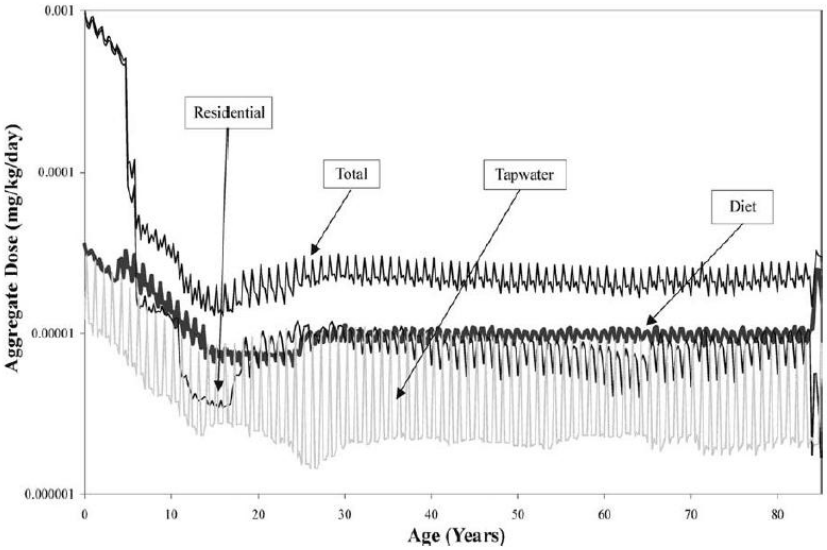

Figure 1. Average daily doses of Alpha by age and season for the modelled population. The doses include the average dose for tapwater, diet, and residential sources as well as the total (aggregate) dose for all routes.

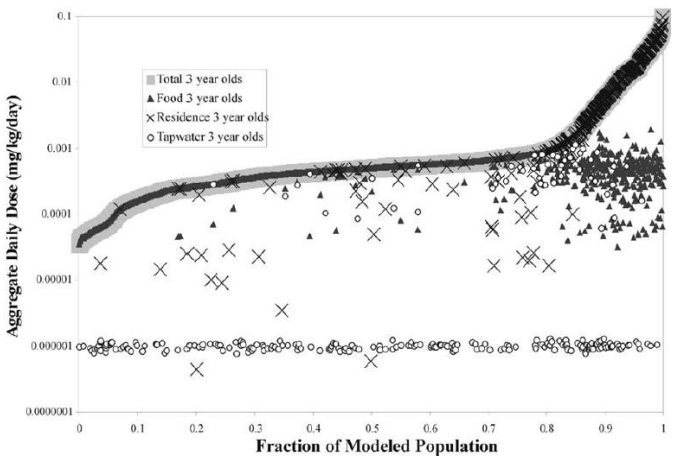

Figure 2. This figure presents each individual's total (aggregate) dose and the dose from tapwater, residential and dietary sources during winter for individuals aged three. The data for each individual have been ranked according to total dose. For almost all of the individuals the total dose is dominated by one source of exposure. Thus the symbol for the dominant source specific dose falls close to or on top of the symbol for aggregate dose. Residential exposure is the dominant source for individuals with high aggregate doses.

Discussion

The results of the modelling are a function of the specific assumptions developed for the three pesticides and may not apply to other compounds. However, the results demonstrate how the model can give insights into the doses and associated risks that result from the exposure to each pesticide both singly and cumulatively.

The results of the model runs presented in Figure 1 suggest that magnitude of the average daily aggregate dose in a season and the sources of those doses for the modelled pesticides are strongly affected by age and to a lesser extent season. The largest doses occur to children and occur from residential sources. After age six the dominant source of the average daily aggregate dose is dietary exposures. Tapwater exposures do not make a significant contribution at any age.

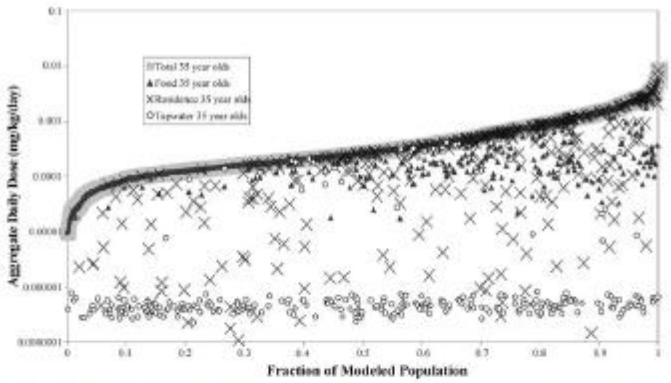

Fig. 3. This figure presents each individual's total (aggregate) dose and the dose from tapwater, residential and dietary sources during winter for individuals aged thirty five. The data for each individual have been ranked according to total dose. Both diet and residential are dominant sources for individuals with the high aggregate doses.

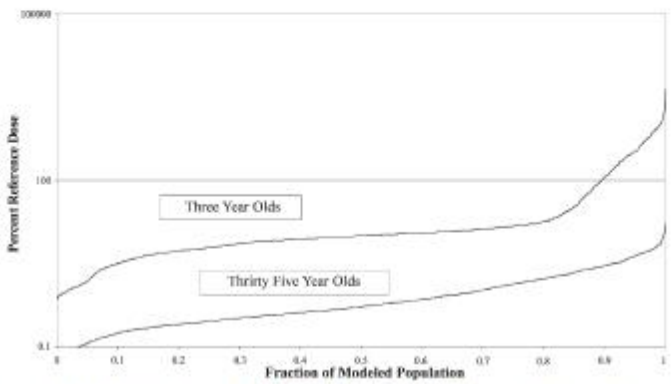

Fig. 4. Estimates of %RfD for the 2000 modeled individuals at ages three and thirty five during winter. Estimates have been separately ranked. Approximately 10% of the three year olds have %RfD that are greater than 100. None of the 35 year olds have %RfD that are greater than 100.

The limited effect of tapwater occurs in part because this source affects such a small portion of the population.

The dietary and tapwater sources of pesticide exposures for Alpha vary by season. This can be seen in the cyclical pattern in the estimates of dose from each source in Figure 1. The seasonal variation in the tapwater is driven by the seasonal nature of tapwater contamination, see Table 4. The seasonal variation in diet is created by the seasonal pattern of consumption (more apples are consumed in the fall and winter than in spring and summer).

Figures 2 and 3 indicate that all of the simulated individuals have at least some exposure to Alpha on at least one day of the winter season when they were aged 3 and 35. This is the probable result of Alpha residues being found in apples, which either directly (raw) or as a component of food forms (juice, pies, pastries, etc.) occur in many individual's diets. The range of maximum seasonal dose is quite large, ranging about three orders of magnitude across the population. As would be expected, the dietary doses for the 3 year old children are higher than the doses for the 35 year old adults.

The sources of exposure for the highly exposed portions of the populations differ in the two age groups. For the three year olds, the top 10% of aggregate doses occur in individuals whose aggregate doses are dominated by residential exposures. In the 35 year old's, the top 10% of the population includes individuals whose aggregate doses are dominated by both residential and tapwater sources.

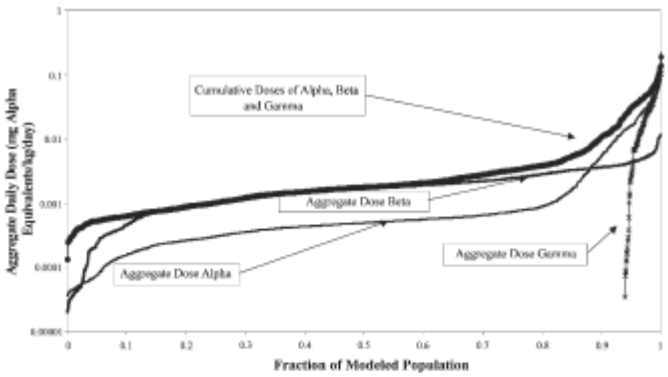

Fig. 5. This figure presents the maximum seasonal aggregate doses of Alpha, Beta, Gamma and cumulative dose of all three pesticides during winter for individuals aged three. All four dose distributions have been ranked separately. Units of dose are in mg of the index pesticide (Alpha).

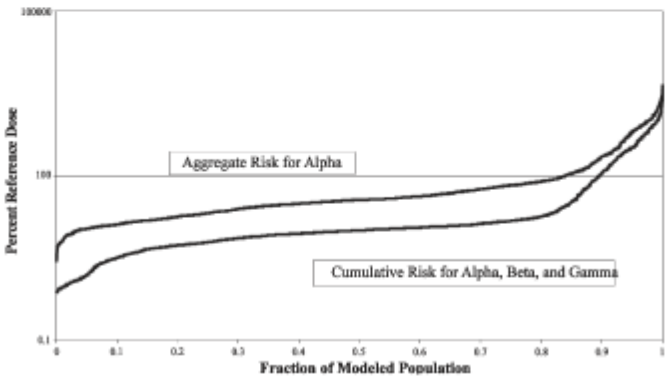

Fig. 6. This figure presents the %RfD associated with each of 2000 Modeled individual's aggregate exposures to Alpha and cumulative exposure for Alpha, Beta, and Gamma in three year olds during winter. Both distributions are ranked separately.

In Figure 4, the aggregate doses for Alpha for 3 year olds in winter result in %RfDs that are greater than 100 (for at least one day per season) for about 10% of the population. A comparison of Figs. 2 and 4 demonstrates that both residential and dietary sources resulted in individuals having %RfD values greater than 100. In contrast none of the thirty five year olds have doses that result in values of %RfD that are greater than 100. This difference is due to the lower doses in adults and because the FQPA factor is applied to both male and female three year olds but only to female 35 year olds.

Figure 5 presents the distribution of interindividual variation of the population's cumulative doses of the three pesticides as well as the aggregate doses for each of the pesticides during winter at age 3. All doses are expressed in units of mg

TEQ/kg/day. The figure demonstrates that exposures to Gamma are limited to a small fraction of the population. This occurs because not all 3 year olds will reside in houses where Gamma is used. In contrast, almost all three year olds had some level of exposure to Alpha and Beta because of the use of the compounds on wheat and apples.

Figure 6 presents the MOE and %RfD associated the aggregate dose of Alpha and the cumulative doses of Alpha, Beta, and Gamma for three year olds in the winter. This figure indicates that approximately 10% of three year olds had %RfDs in excess of 100 from exposure to Alpha for at least one day per season and the fraction with %RfDs increased to 16% when the cumulative risks were determined. Thus the net effect of considering cumulative risk was an increase of 6% in the population potentially of concern.

Conclusions

Since the three pesticides in this analysis are similar to actual pesticides, a number of conclusions can be drawn that may be relevant to other pesticides. First, children's dietary exposures are expected to be higher than adults when the residues occur on foods that are consumed by both children and adults (apples and wheat). This finding is based on the fact that dietary intakes are higher for children on a weight basis than adults.

Second, when the sources of pesticide are independent, aggregate exposures for most individuals in the general population will tend to be dominated by a single route of exposure. It should be noted that this analysis focused on the general population and did not investigate specific subpopulations where concurrent exposures would be more likely to occur (such as farm families or agricultural workers).

Third, certain sources affect all individuals in a population while other sources only affect a small subpopulation. As a result, cumulative plots of dose will typically appear as a series of steps and the frequency distribution of doses (not shown) will be multi-modal.

In summary, this paper demonstrates that probabilistic models such as the one used in this paper using data that are currently available for pesticides can characterize both cumulative and aggregate exposures. The specific model used in the paper can be used to identify the critical sources of exposure and the influence of factors such as age and season. For example, the findings in this analysis suggest

that the reduction of risks from the three hypothetical pesticides should focus on the residential sources of exposure.

Electronic Databases

Agricultural Research Service, Beltsville Human Nutrition Research Center, Food Surveys Research Group, US Department of Agriculture, 1991. *Continuing Survey of Food Intakes by Individuals, 1989–1991.*

Bureau of the Census, US Department of Commerce, and Bureau of Labor Statistics, US Department of Labor, 1999. Current Population Survey, Annual Demographic Survey — March Supplement, 1992–1994 and 1996–1999 Data Sets.

Bureau of the Census and US Department of Housing and Urban Development, 1993. *American Housing Survey*.

National Center for Health Statistics, Centers for Disease Control and Prevention, US Department of Health and Human Services, 1996. *1996 Natality Data Set*.

National Center for Health Statistics, 1994. *1988– 1994 National Health and Nutrition Examination Survey (NHANES III)*.

US Environmental Protection Agency, 1992b. *National Home and Garden Pesticide Use Survey*. Prepared by the Research Triangle Institute for the Office of Pesticides and Toxic Substances, Biological and Economic Analysis Branch.

US Environmental Protection Agency, Human Exposure and Atmospheric Sciences Division, Human Exposure Research Branch, 1994. *National Human Activity Pattern Survey*.

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References

Anon. Exposure factors handbook. EPA/600/P-95/002F(A–C). Washington (DC): US EPA (U.S. Environmental Protection Agency), 1997.

Goodrum PE, Diamond GL, Hassett JM, Johnson DL. Monte Carlo modelling of childhood lead exposure. Development for use with the USEPA IEUBK model for lead in children. Hum. Eco. Risk Ass. 1996; 4(2):681–708.
Harrington NW, Curry CL, Carlson-Lynch HJ, Price PS. The microexposure event modelling approach to probabilistic exposure assessment. In: Air and Waste Management Annual Meeting, San Antonio, Manuscript A443, May, 1995.
ILSI. In: Mileson B, Faustman E, Olin S, Ryan P, Ferenc S and Burke T, editors. A framework for cumulative risk assessment. An ILSI risk science institute workshop report. Washington (DC): ILSI; 1999.
Keenan RE, Henning M, Goodrum P, Gray M, Sherer R, Price P. Using a microexposure Monte Carlo risk assessment for dioxin in Maine (USA) fish to evaluate the need for fish advisories. In: Proceedings Dioxin '93 — the Thirteenth International Symposium on Chlorinated Dioxins and Related Compounds, Vienna, Austria, 1993.
McKone TE, Ryan PB. Human exposures to chemicals through food chains. An uncertainty analysis. Environ. Sci. Technol. 1989; 23(9):1154–63.
McKone TE, Daniels JI. Estimating human exposure through multiple pathways from air, water, and soil. Reg. Toxicol. Pharmacol. 1991; 13(1):36–61.
Morgan MG, Henrion M. Uncertainty: a guide to dealing with uncertainty in quantitative risk and policy analysis. New York: Cambridge University Press, 1990.
Muir WR, Young JS, Benes C, Chaisson CF, Waylett DK, Hawley ME, Sandusky CB, Sert Y, DeGraff E, Price PS, Keenan RE, Rothrock JA, Bonnevie NL, McCrodden-Hamblin JI. A case study and presentation of relevant issues on aggregate exposure, In: Olin S, editor. Aggregate exposure. A report to EPA/ILSI workshop. Washington (DC): ILSI Press; 1998.
Price PS, Curry CL, Goodrum PE, Gray MN, McCrodden JI, Harrington NW, Carlson-Lynch H, Keenan RE. Monte Carlo modelling of time-dependent exposures using a microexposure event approach. Risk Anal. 1996; 16(3):339–48.
Price P, Sample J, Strieter R. Determination of less-than-lifetime exposures to point source emissions. Risk Anal. 1992; 12(3):367–82.
Price PS, Chaisson CF, Young JS, Christensen C, Doyle E, Suhre FB. Background document for the session: review of an aggregate exposure assessment tool. FIFRA Scientific Advisory Panel, The LifeLine™ Project to model Aggregate Exposures to Pesticides, Arlington, VA, September 22, 1999.
Shurdut BA, Barraj L, Francis M. Aggregate exposures under the Food Quality Protection Act. An approach using chlorpyrifos. Reg. Toxicol. Pharmacol. 1998; 28(2):165–77.
US EPA (US Environmental Protection Agency). Guidelines for exposure assessment. Fed Reg. 57(104), 1992a.

US EPA. National home and garden pesticide use survey. Prepared by the Research Triangle Institute for the Office of Pesticides and Toxic Substances, Biological and Economic Analysis Branch, 1992b.
US EPA. Analysis of Human Activity Pattern Survey (NHAPS) Office of Research and Development, July 1996. Final Report EPA 68-01-7325.
US EPA. Draft Standard Operating Procedures (SOPs) for residential exposure assessments, 1998.

Chapter 3

A conceptual framework for modelling aggregate and cumulative exposures to chemicals

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Abstract

Computer simulation programs have been identified as useful tools for characterizing uncertainty and variability in longitudinal exposures to multiple sources by multiple routes of exposures. This paper provides a conceptual framework for such programs that separates and appropriately models the processes that determine uncertainty, inter- and intraindividual variability, as well as the processes that determine the relationships between the individuals and sources of exposure. The framework is based on a series of four nested loops. These are: the exposure event loop that models the route-specific doses to a person from one or more sources at one point in time; the time step loop that moves a person through time updating the sources and the person's characteristics, the interindividual variation loop that determines the initial characteristics of each person modelled, and finally the uncertainty loop that characterizes the uncertainty from model and parameter uncertainties. This framework provides a flexible and internally consistent approach for the design of simulation software.

Keywords: aggregate, cumulative, simulation, software.

Introduction

Over the last 10 years, there has been a growing recognition of the need for tools to assess exposures to multiple chemicals from multiple sources. This need has been driven by legislative and regulatory actions such as the 1996 Food Quality Protection Act (FQPA), the residual risk portions of the 1991 Clean Air Act, and the Voluntary Children's Chemical Exposure Program (EPA, 2000a).

There has been a general recognition that computer simulation software (software) is a useful tool for these assessments (ILSI, 1998, 1999; EPA, 1999, 2000b, 2001). The goal of such software has been to characterize one or more of the following:

- doses from exposures to multiple chemicals;
- doses from exposures that occur by multiple routes from a source of exposure;
- doses from exposures that occur from multiple sources;
- variation in doses received by a person over time (intraindividual variation);
- variation in doses across a population (interindividual variation); and
- uncertainty in predicted doses.

This paper proposes a conceptual framework for the construction of simulation software that achieves these goals. This framework is based on the concept that models must place the concept of a ‘‘person’’ at the centre of the design. For this reason, the framework is often referred to as ‘‘Person Oriented Modelling’’ or POM. The framework is based on a series of four nested loops: the exposure event loop, the time step loop, the interindividual variation loop, and the uncertainty loop.

The framework presented in the paper is not entirely new. Many software programs such as microexposure air programs, dietary software, and the aggregate and cumulative software programs developed to meet the needs of the FQPA, use approaches that are consistent with portions of the framework; however, no existing software completely incorporates the framework and the framework has not been clearly described in the literature.

The framework is presented in the following section. Technical issues related to the framework are presented thereafter. Where possible, existing software programs that are consistent with the framework are identified. In this paper, the term ‘‘model’’ refers to a conceptual model and ‘‘program’’ refers to an actual computer program.

Description of a Framework for Simulating Exposures

Modelling people not sources

The development of software to characterize concurrent exposures to widely different sources of multiple chemicals leads inexorably to a person- or receptor-oriented designs (Price *et al*., 1996a; Muir *et al*., 1998; Zartarian *et al.,* 2000; CLA, 2002). These designs focus on the population of interest rather than the sources of exposure. To understand the impact of the change of focus from source oriented to person oriented, it is useful to review the conceptual model that historically has governed exposure assessments, source to- dose modelling.

Source-to-dose modelling begins at the point that a chemical enters the environment, moves the chemical through the various media, and models the amount and rate of a chemical enters a person, see Figure 1. Source-to-dose models have been used in regulations that set emission standards under the Clean Water and Clean Air Acts, clean up standards for hazardous waste sites, and tolerances for chemical additives in food. The widespread use of these models occurs for two reasons. First, regulatory agencies place a high priority in defining the relationship between a specific source and the resulting doses in the affected

population because the purpose of most regulatory programs is the control of specific sources. Second, by following a chemical from a source to a final dose, the researcher is forced to systematically address all of the processes that determine the movement of a chemical from the source to the exposed person.

The weakness of this approach is that the characteristics of the source define the starting point of the model and to a large extent determine the design of the subsequent portions of the model.

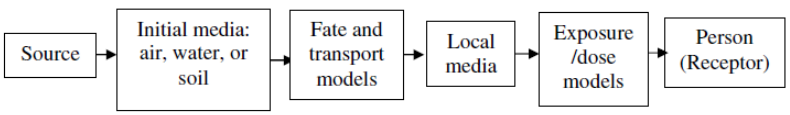

Figure 1. Source-to-dose models.

This dominance causes problems when the goal of the assessment is to determine the total exposure to a chemical or group of chemicals. Software that assesses aggregate exposures (total dose a substance received from multiple sources) and cumulative exposures (total doses of multiple substances received from multiple sources) must address multiple, independent, and diverse sources of exposure (see Figure 2). This creates a need for consistency across the different source-to-dose models. Consider a program that simulates the doses from two independent sources where both sources require the determination of a chemical's concentration in a microenvironment, such as a backyard. If one module of the software assigns the person to an urban town house with a small yard and a second assigns the person to a rural home with a large yard, then the predictions of the total dose are not likely to be a realistic description of an actual person. Similar problems occur with assumptions concerning the person's age, weight, or the season when the exposure occurs. EPA refers to this as the need for consistency in the ''temporal, spatial, and demographic characteristics'' of persons within the population (EPA, 1999). This need for consistency of assumptions for the characteristics of the modelled person and the microenvironments the she or he interacts with leads to the development of person-oriented model designs and moves the ''sources of exposure'' to the periphery of the model design.

Modelling exposures from multiple sources, by multiple routes, and multiple chemicals

An application that demonstrates the first two loops of the framework, the exposure event loop and the interindividual loop, is given in Figure 3, a flow chart for a multi-route, aggregate, and cumulative software program. In this example,

only two chemicals are assessed; however the approach can be extended to any number of chemicals.

The exposures in this example are assumed to occur over a period of time sufficiently short that; (1) when determining the toxicological effects of the exposures, the doses of a chemical from each source can be treated as a constant dose for the duration of the period of time; (2) the levels of each chemical in the relevant microenvironments can be treated as constants, and (3) the person's exposure-related characteristics (body weight, breathing rate, tap water consumption, location, etc.) can be treated as constants. This period may be as short as a few seconds, or as long as a year, depending on the toxicological end point of interest, the person, and the sources of exposure (Price *et al.*, 1996a).

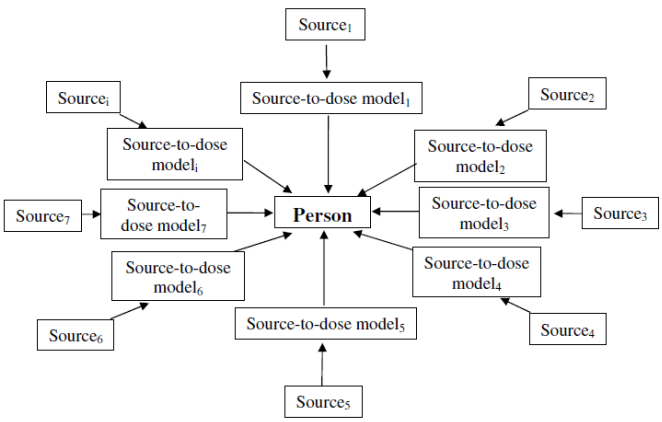

Figure 2. Multi-source exposure modelling.

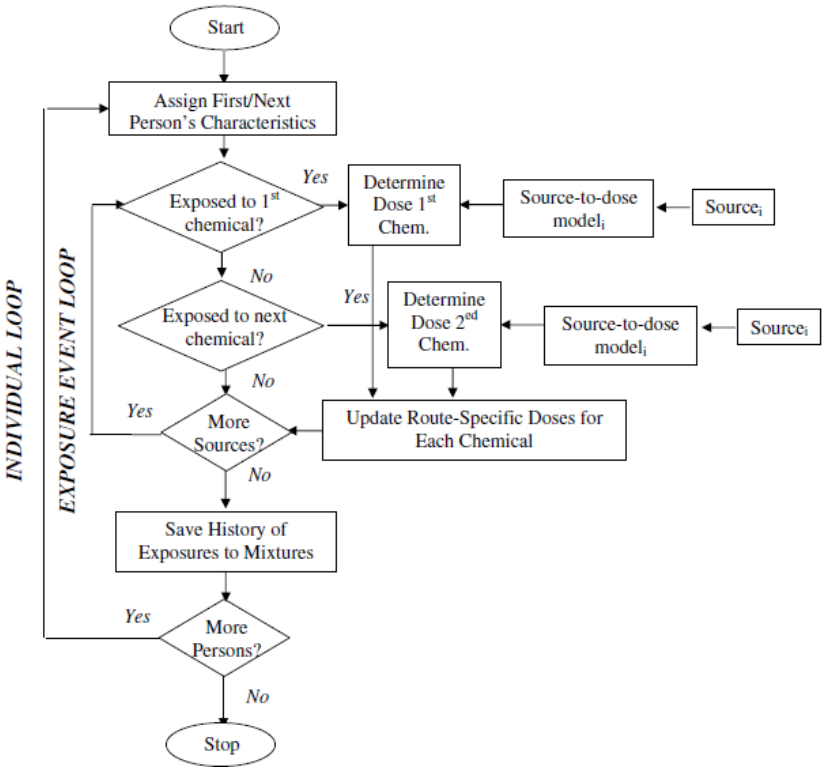

Figure 3. Flow chart for a model of interindividual variation in exposures to multiple chemicals – one time step.

As Figure 3 indicates, the program begins by determining the exposure-related characteristics of the first person being simulated in the software. These characteristics are those parameters required to determine the doses from the various sources that are related to the person and which influence the dose estimates for more than one source. The parameters could include information on demographics, housing, activities, relevant microenvironments, and the individual's physiology.

The assignment of the values for these characteristics is done by sampling from distributions that reflect the interindividual variation of the population of interest. For example, if the population is defined as pregnant women, then the age-specific pregnancy rates can be used to assign the age for the women being simulated. If the population is defined as persons living in a specific county then local demographic information can be used to assign a distribution of characteristics for the population.

Once the data for the person are determined, the program enters the exposure event loop. In this loop the program determines the probability of being exposed to each of the potential sources of the chemicals. This potential is conditional on the characteristics of the person. For example, if a person is one year old there is a higher chance that she or he will have an oral exposure to a phthalate plasticizer used in toys than if she or he is 25 years old. Women are more likely to have acetone exposure from use of nail products than men. Children living in houses with large lawns have a greater chance of exposure to residential lawn products than children living in multi-storey apartments.

The decision of whether a person is exposed is made independently for each source of each chemical. This allows the assessor to characterize when exposures to different sources of the same or different chemicals will and will not co-occur. For example, an assessor may wish to assume that person who rides a bus to work on a given day will not commute in a car. Thus, in an assessment of benzene exposure, the determination of benzene exposures from commuting in a car versus in a bus can be set up as mutually exclusive. Where the exposures are associated with one another the decision that one exposure occurs can be used to increase the probability of exposure to a second source. Thus, a glycol ether exposure from a floor stripper (wax remover) can b e linked to an exposure from a subsequent application of a floor wax. This process also applies to the co-occurrence of different chemicals. Exposure to benzene can be modelled to co-occur with exposure to butadiene when they have a common source (tailpipe emissions). Exposures to two pesticides in competing products (that control the same pest) can be made mutually exclusive. This ability to make conditional decisions on whether a person is exposed is a critical component of the proposed framework.

Once the decision has been made that the person has been exposed to a specific source of a chemical, then a source-to-dose model is used to determine the dose for each of the relevant routes of exposure. Unlike software that is based on traditional source-to-dose models, the characteristics of the source, the transport processes, and the exposure calculations can be made contingent on the characteristics of the person being assessed. This allows the software to maintain consistency in these characteristics across the various source-to-dose models.

If the exposure occurs, then the doses from the exposure to the source are determined. Exposure to a source may result in doses that occur by a single or multiple routes (oral, dermal, and inhalation) of exposure. The estimates of the doses from each of the relevant routes of exposure are not combined at this step in the simulation but are saved by route. Once the various doses are saved, the

program moves to the next source (another cycle in the exposure event loop). If the exposure to a source does not occur, then the framework proceeds to the next source. This program continues until all of the sources for all of the chemicals have been evaluated.

The doses produced in one exposure event loop can be used in a variety of ways. The doses from each source can be summed to give total route-specific doses for each chemical in the mixture to which the person was exposed. In addition, the doses can be segregated by source to give the source-specific doses. The route-specific doses can be used:

1. As inputs to route-specific risk characterization models (using the relevant toxicological benchmarks for the oral, inhalation and dermal doses);
2. Used to estimate the total dose and used in non-route specific models of risk;
3. used in models of cumulative risk for exposure to mixtures; or
4. used as inputs to physiologically based pharmacokinetic (PBPK) models of organ specific doses in the person

The source-specific doses can be used to determine the relative source contribution of each source.

Once the determination of the first person's exposures is complete, the program leaves the exposure event loop, returns to the beginning of the program, and selects another person. This return creates the second type of loop in the framework, the individual loop. In this loop, the characteristics of this new person are again selected based on data on the interindividual variation of persons in the population of interest. Once these values are assigned, the exposure event loop is re-entered. The program continues to cycle through the individual loop until the desired number of individuals has been simulated. The outputs of this process are sets of route- and source-specific doses for each chemical for each of the simulated persons in the model run. This set of doses characterizes the interindividual variation in the dose(s) of a chemical or a mixture of chemicals across the population for a specific duration at a specific point in time.

A number of software programs use this approach to estimate daily doses of pesticides and chemicals from dietary and air exposures. Dietary software such as DEEM™ (Barraj *et al*., 2000), and the dietary portions LifeLine™ (The LifeLine Group, 2002), and CARES™ (CLA, 2002) are software that follow this approach. When assessing a daily dietary dose, these programs pull a dietary record for one person from the United States Department of Agriculture's Continuing Survey of

Food Intakes by Individuals (CSFII). The CSFII record contains the name and amount of each food item a person consumes on a day. Using this record and a list of foods that have pesticides residues, the programs go item by item through the daily diet (the exposure event loop). For each food item, the program checks to see if there was a residue on that food item. If there is no residue, the program goes on to the next food. If there is a residue, then the program determines what is the concentration of the residue and what is the dose from consuming the food item. At the end of the list, the total oral dose of pesticide from all foods is determined and given as an output of the daily dose. This process is repeated with other records (the individual loop).

Microenvironmental software programs track persons through a series of microenvironments and determine the total air exposure. Examples of such programs include pNEM (Law *et al*., 1997); SHAPE (Ott, 1981); CPIEM (Rosenbaum *et al*., 2002). A similar approach also is used in SHEDS for inhalation exposure (EPA, 2002a). These software programs begin by selecting a record of the activities of one person over a single day from the results of surveys of human activities such as the National Activity Pattern Survey (Klepeis *et al*., 1996), or from the Consolidated Human Activity Database (EPA, 2002b). These records consist of diaries of one person's daily activities that specify the microenvironments the person occupied during a day (bedroom, kitchen, car, office, etc.), a description of what activity was performed in that microenvironment, and the duration of the activity. These programs then select the first microenvironment and determines the inhalation dose given the person's breathing rate, duration of time in the microenvironment, and the level of contaminant in the air of the microenvironment. Once the dose is determined, the program moves to the next microenvironment (the exposure event loop). This process is repeated until all microenvironments are completed. The total inhalation dose is then determined for the day. This is repeated for each person in the population of interest (the interindividual loop). EPA has used this approach to estimate cumulative exposures to organophosphorous pesticides (Price *et al.,* 2002; EPA, 2002c).

Modelling longitudinal exposure

In the above examples, the exposures are assumed to happen during a single point in time. Over longer periods of time, inputs to the source-to-dose models cannot be assumed to be constant. In addition, exposure to specific sources will begin and end at specific times. As a result of these changes, a person's exposures will vary over time.

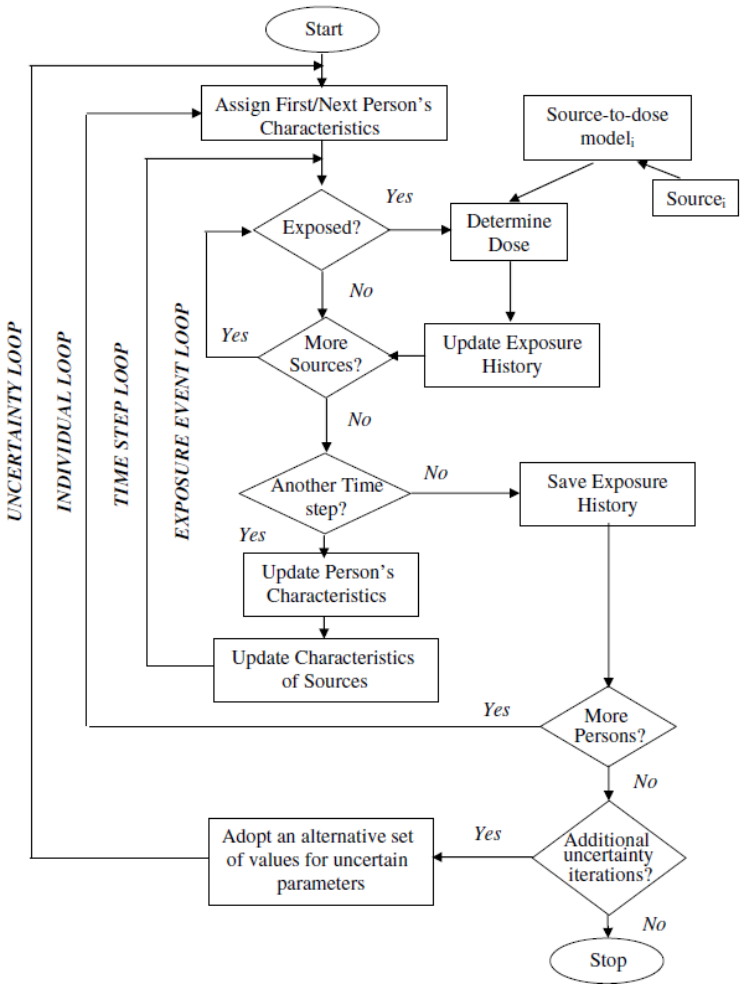

Figure 4. Flow chart for the complete framework consisting of four loops: uncertainty, individual, time step, and exposure event.

Figure 4 presents a flow chart for a longitudinal software program that determines how individuals' exposures change over time. (Note, to simplify the presentation the flow chart only addresses a single chemical. As discussed above, the framework can be extended to any number of chemicals.)

Modelling longitudinal exposures is achieved with an additional loop called the ''time step loop''. The time step loop occurs between the exposure event and the individual loops. As Figure 4 indicates, the program again begins with a definition

of the person's characteristics; however, now the characteristics are those that apply to the first time step. The program then enters the exposure event loop and the exposures from each exposure source are modelled. As discussed above, the period of time that applies to the exposure event loop is assumed to be sufficiently short that all of the inputs to the exposure-to-dose models can be viewed as constants. Once each source of exposure is modelled, the program determines if there is an additional time step to be modelled. If there is an additional step, the characteristics of the person, the microenvironments, and the sources of exposure are modified to reflect changes that occur between the two time periods. Once the characteristics are revised, the program then returns to the beginning of the exposure event loop to determine the doses that occur to the individual in the new time step. This returning back and revision of the time-sensitive characteristics is the time step loop. If there are no additional time steps to be assessed, the program moves to the next person (the individual loop).

There are a number of methods for modelling longitudinal exposures in individuals who have been adopted by different modellers and the area is the subject of ongoing research. A full discussion of the approaches is beyond the scope of this paper; however, the proposed framework provides support to longitudinal models in two ways. First, the framework provides the longitudinal models a rich definition of the time variant and invariant characteristics such as the nature of the microenvironments, day of the week, and season of the year.

Second, the framework is modelled a series of chronological time steps. By sequentially modelling the time steps, the framework is able to use the characteristics of the person, microenvironment, and sources at one time step to predict characteristics at the subsequent time step. This allows the modeller to capture known temporal dependencies. For example, body weight would not be expected to significantly change from day to day but would change over ages 0 to 18. Painting a room in a home will result in a release of glycol ether on the day that the paint is applied and on subsequent days.

The output from this longitudinal model is an ''exposure history'' for each of the modelled persons. The exposure history consists of the route- and source-specific doses for each chemical for each time step. The exposure histories of multiple persons allow the characterization of both inter- and intraindividual variation. This approach has been used in a number of longitudinal models (LifeLine™ (The LifeLine Group, 2002), CARES™ (CLA, 2002), SHEDS (EPA, 2002a), APEX (EPA, 2003), and Calendex™ (Novigen, 2000).

This output of the time step loop can be used in a number of ways. First, the data can be averaged to give estimates of the average dose over longer periods of time. Thus, if the time step was a single day and if the model is run for 365 days, then the daily doses can be averaged to give a prediction of the person's annual average dose. Second, the highest daily dose in a given year can be determined by ranking the 365 daily doses for a single simulated person. This ''peak'' annual daily dose can be used to evaluate acute risks that the person receives over time (Price *et al.*, 2002). This peak exposure can be useful in the evaluation of events that happen infrequently such as painting a room or staining a deck.

Third, the exposure histories can be used as inputs to PBPK models. Such models can be used to predict the time course of internal doses that occur because of total exposures. These internal doses can be used to estimate doses to the reproductive organs and the foetus. Because the data are longitudinal exposures for a specific person, the cumulative impact of chemicals, their metabolites, and cellular damage can be modelled (LifeLine, 2005). Because exposure histories are available for each person, the interindividual variation in such measures of cumulative impact can be modelled.

Modelling uncertainty

Up to this point, the discussion of the framework has been limited to variation, interindividual variation in selecting a person's characteristics and intraindividual variation in the person's characteristics over time. Uncertainties in the inputs and their impact on the resulting dose estimates have not been discussed.

The estimates of exposure produced by this framework are subject to a number of uncertainties. Inputs to simulation programs are subject to uncertainties from sampling bias, limitations in analytical methodologies, or limited sample size (Morgan and Henrion, 1990). In addition, use of specific algorithms results in additional uncertainty (modelling uncertainty) (EPA, 1992). Finally, there are unknown sources of uncertainty (surprise) (Hammit and Shlyakhter, 1999).

Modelling cannot be performed for all sources of uncertainty. However, uncertainty in inputs and in algorithms can be characterized in the framework using a fourth loop called the uncertainty loop. Figure 4 presents a flow chart for a software program similar to that in Figure 3 but where uncertainty is quantitatively modelled in an outer uncertainty loop. In this outer loop, the framework adopts alternative analyses of exposure that reflect uncertainty in model inputs and model algorithms. For example, if the distribution of air concentrations in homes is described by a lognormal distribution with an uncertain mean, alternative values

for the mean can be specified in the outer loop. The approach of using an outer loop to model uncertainty has a long history in simulation software (Bogen and Spear, 1987; Hoffman and Hammonds, 1994; Price *et al*. 1996b). The approach is also used in SHEDS (EPA, 2002a).

The output of this joint model of uncertainty and variation is an uncertainty distribution around each of the outputs of the framework described in the previous section. Thus while the model described in Figure 3, can provide an estimate of the 95th percentile for an annual average exposure to persons in a population, the addition of the uncertainty loop allows the determination of confidence limits on that estimate that reflect specific sources of uncertainty

Technical Issues in the Application of the Framework

Defining the population

Characterization of interindividual variation begins by assigning sets of values that reflect the variation in the values occurring in the population of interest. This requires that the software begin with a clear definition of the target population. Without a clear description of the population that is being assessed, it is possible to bias the assessment and misuse data. Populations can b e defined in three ways; in terms of the specific sources of exposure included in the assessment, the availability of data, or the use of a standard predefined population.

As discussed above, the population in source-to-dose models is the population receiving a dose from the source. Examples of this approach include consumers who use a product or persons living downwind or downstream of a point source. This approach raises some difficulty in that such populations may differ from the general population and, thus, data on variation taken in the general population may not be relevant. For example, householders with gardens tend to be older than the general population of homeowners (USEPA, 1992). Thus the use of data on the number of small children in homes in the general population will overestimate the frequency of small children present in homes with gardens. Defining the population in terms of an exposure source becomes unwieldy when multiple sources of exposure are being modelled. Consider an assessment that evaluates three phthalates and uses the exposures to the chemicals as the basis for the population to be modelled. One phthalate is used in cosmetics, the second in children's toys, and the third in food packaging (ATSDR, 1990, NCEH, 2003). Each phthalate has a distinct exposed population (toys - small children, cosmetics - older children and adults, food containers - all ages) and have some potential for overlap (small children passively come into contact with makeup on adults, some young

children actively use makeup, some parents handle toys, and all consuming food packaged with the phthalate). Such cumulative populations become very difficult to describe or to model.

The alternative approach is to define the population in terms of some independent demographics. Examples might include, the general US population, all persons living in town houses, workers in a specific SIC code, or all 3-year-old children. Under this approach, persons in the population are included in the software whether or not they are actually exposed to one or more sources and the program determines if they are exposed or not exposed. If they are not exposed, the program predicts a dose of zero for the persons. The result is a prediction of the distribution of doses in a population that includes some fraction of the population having zero doses.

This approach has a number of advantages. First, it allows the use of national demographic data for the population. Second, the program can be extended to new sources of exposure. The drawback to this approach is that it wastes the computer's time in selecting and evaluating nonexposed persons. However, tailoring the program to the demographic groups with the greatest potential for exposures can minimize this drawback. For example, if the exposure is associated with swimming in a home pool, the program can be limited to homeowners with pools.

A third approach is to use a predefined population that is based on one or more major surveys of exposure related information. CARES™ has created a ''reference population'' of 100,000 persons who have detailed information on their properties specified. The population is designed to be representative of the US population (Sielken and Holden, 2001). In Calendex™ all adults are based on the persons who participated in the 1994–1996, 98 Continuing Survey of Food Intake by Individuals (Novigen, 2000). SHEDS is based on the persons with activity patterns in CHADS database of activity patterns (EPA, 2002a). The drawback to this approach is that subpopulations of interest may not be well represented in such reference populations.

Modelling correlation and autocorrelation

A major concern in defining inter- and intraindividual variation in exposures is the correlation among values that occur for different characteristics. Failure to capture correlations can affect the estimates of the distributions of dose and risk. There are two approaches to defining correlations. The first approach is to rely on data taken from a record of data taken from a single person (a record-based approach).

Record-based approaches capture the correlations between the values of a person's exposure characteristics since the data come from a single person. Record-based approaches have been used for the evaluation of dietary and human activity pattern-based assessments.

There are, however, drawbacks in relying entirely on a record-based approach. First, while surveys have been performed of human activities and diet, no survey has been performed that captures all of the behaviours that are associated with chemical exposure (use of consumer products, hobbies, home heating system, room sizes, or air exchanges rates). In addition, it is difficult to obtain survey results on a person's behaviours for periods of time longer than one or two days. Therefore, strict reliance on record-based approaches cannot be used to investigate exposures that occur over longer periods of time then a few days. Because of these limitations many software programs cannot rely on a strict record-based approach.

Modelling the person, family structure, and residence

The characteristics of the person go beyond data on activities and physiology. The person's potential for exposure will require defining the person's residence and family structure. The reason for this is that the potential for exposure will be affected by both the characteristics of the residence and the activities of other persons in the home.

The characteristics of a home have a great influence on the potential exposure. Parameters that should be considered in the model included room sizes and air exchange rates, the relationships between room locations, heating systems, and the presence of attached garages, workrooms, and home offices.

The family structure has a great influence on exposures. Parents of infants in their homes have different activity patterns, diets, and uses different products then adults without children. Thus, the model may be required to assign a family structure to the person (other adults, children, infants, etc) present in the home. Modelling family and other personal interactions is an open-ended task. Therefore it is critical that the user define the sources that will be modelled and limit the modelling of other individuals to those events that affect the person's exposures to those sources.

Management of outputs

A drawback to the proposed approach is the amount of data that would be generated. Consider the following scenario: An analysis of inter- and intraindividual variation is performed and generates estimates for 1000 children. The software determines inter- and intraindividual variation of daily doses of five chemicals over the 2-year period from the children's second to fourth birthdays. This requires calculating estimates for 730 days (2x365 days). If the exposures occur by all three routes, then for each day there are three doses for each of the five chemicals. The total number of doses modelled is:

$1000 \times 730 \text{ days} \times 3 \times 5 = 1.1 \times 10^7$

If the uncertainty in the estimates is evaluated then an additional 500 iterations of the uncertainty loop are performed.

$1.1 \times 10^7 \times 500 = 5.5 \times 10^9$

As this demonstrates, even a modest number of iterations for uncertainty and variation can result in very large data outputs. These outputs result in large files and long run times.

One approach that can assist in this problem is to decide prior to the assessment which outputs are of interest and save only those outputs that are of interest. For example, if the analysis of interest is annual exposures then the model can be instructed to average the 730 days into two annual doses. This would reduce the output files 365 fold. If confidence limits are needed on the mean and the 95th percentile of the variation in the population, then the mean and the 95th percentile of the simulated population can be determined as part of the uncertainty loop and saved. This will reduce the output to 1000 outputs (500 estimates of the mean and 500 estimates of the 95th percentile). The drawback to this approach is that the model will have to be rerun if alternative measures are required.

Summary

The framework described in this paper, while simple, has the ability to model doses with duration ranging from acute to lifetime and for ages varying from conception through birth to any age. The approach can be extended to any number of chemicals, sources of exposure, and routes of exposure. The approach allows for the correct separation inter- and intraindividual variation and

uncertainty. Finally, the proposed approach is consistent with many models currently in use or under development by EPA, academia, and industry.

Person-oriented modelling rather than source oriented modelling provides a number of advantages. First, it is the risks to persons and how they change as a function of sources and mitigation that is the ultimate goal of the model. Second, inter- and intraindividual variation in dose and risk are key outputs for the model. Thus, the model should begin with a specific focus on inter- and intraindividual variation. Third, defining internally consistent doses from exposure to multiple sources and routes of exposure to one chemical or to multiple chemicals can only occur if the same person is modelled for each source. Focusing the modelling on the person allows for the calculation of internally consistent models of the person's exposures with regard to time, location, age, and source. Finally, focusing on the person allows the model to track each person over time (from the characteristics of the parents at conception, the characteristics of the mother during pregnancy, and the characteristics of the person from birth to the end of his or her life).

Construction of computer programs based on this framework has the additional benefit of increasing linearly with the number of sources. This is in contract to programs such as decision tree analysis where model complexity and run times increases geometrically. This means that large numbers of sources can be tracked in one model. In addition, adding additional sources to an existing model can be easily performed.

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References

ATSDR. Toxicological profile for Di-n-butyl phthalate, Public Health Service, US Department of Health and Human Services, 1990.

Barraj L.M., Peterson B.J., Tomerlin R.J., and Daniel A.S. Background document for the sessions: dietary exposure evaluation model (DEEM™) and DEEM™ decompositing procedure and software. Presented to FIFRA Scientific Advisory Panel (SAP) by Novigen Sciences, Inc., 29 February 2000.

Bogen K.T., and Spear R.C. Integrating uncertainty and interindividual variability in environmental risk assessment. Risk Anal 1987: 7: 427–436.

CLA. CARES user guide CARES version 1.0. CropLife America, 20 March 2002.

EPA. Guidelines for exposure assessment. Federal Register 57(104), 1992.

EPA. Guidance for performing aggregate exposure and risk assessments. 29 October 1999 US EPA OPP, Washington, DC, 1999.

EPA. Voluntary children's chemical evaluation program. Federal Register, December 26 (65), Num. 248, 81699-81718, 2000a.

EPA. Proposed guidance on cumulative risk assessment of pesticides chemicals having a common mechanism of toxicity. 22 June 2000 US EPA OPP, Washington, DC, 2000b.
EPA. General principles for performing aggregate exposure and risk assessments office of pesticide programs, 2001 (www.epa.gov/pesticides/trac/science/aggregate.pdf).
EPA. SHEDS-wood stochastic human exposure and dose simulation model wood preservative exposure scenario F user's manual. US Environmental Protection Agency Office of Research and Development, National Exposure Research Laboratory. 20 July 2002a.
EPA. CHAD user's guide. Extracting Human Activity Information from CHAD on the PC June 2000, Modified on March 2002 by Systems Development Center Science Applications International Corporation, 2002b, www.epa.gov/chadnet1/reports/CHAD_Manual.pdf.
EPA. Organophosphate pesticides: revised OP cumulative risk assessment, 2002c: http://www.epa.gov/pesticides/cumulative/rra-op/.
EPA. Total risk integrated methodology, TRIM expoInhalation user's document volume I: air pollutants exposure model (APEX, version 3). User's guide. Office of Air Quality Planning and Standards, Draft 24 April 2003.
Hammit J.K., and Shlyakhter A.I. The expected value of information and the probability of surprise. Risk Anal 1999: 19(1): 135–152.
Hoffman F.O., and Hammonds J.S. Propagation of uncertainty in risk assessments. Risk Anal 1994: 14(5): 707.
ILSI. Aggregate exposure. Olin S. (Ed.) A Report to EPA/ ILSI Workshop. ILSI Press, Washington, DC, 1998.
ILSI. A framework for cumulative risk assessment. In: Mileson B., Faustman E., Olin S., Ryan S., Ferenc S., Burke T. (Eds.) An ILSI Risk Science Institute Workshop Report. ILSI Press, Washington, DC, 1999.
Klepeis N.E., Tsang A.M., and Behar J.V. Analysis of the national human activity pattern survey (NHAPS) respondents from a standpoint of exposure assessment. Final EPA Report, EPA/600/R- 96/074 Washington, DC, 1996.
Law P.L., Lioy P.J., Zelenka M.P., Huber A.H., and McCurdy T.R. Evaluation of a probabilistic exposure model applied to carbon monoxide (pNEM/CO) using Denver personal exposure monitoring data. Technical Paper ISSN 1047-3289. J Air Waste Manage Assoc 1997: 47: 491–500.
LifeLine. User Manual Lifeline™ Version 2.0 Software for Modelling Aggregate and Cumulative Exposures to Pesticides and Chemicals, September 30, 2002.
LifeLine. Designing exposure models that support PBPK/PBPD models of cumulative risk. Prepared for USEPA Office Pesticide Programs Under EPA Contract EP-W-04-016 2005. Available at www.epa.gov/scipoly/sap/2004/december3/llgmanuscript.pdf.
Morgan MG, and Henrion M. Uncertainty: A Guide to Dealing with Uncertainty in Quantitative Risk and Policy Analysis. Cambridge University Press, New York, NY, 1990.
Muir W.R., Young J.S., Benes C., Chaisson C.F., Waylett D.K., Hawley M.E., Sandusky C.B., Sert Y., DeGraff E., Price P.S., Keenan R.E., Rothrock J.A., Bonnevie N.L., and McCrodden-Hamblin J.I. A case study and presentation of relevant issues on aggregate exposure. In: Olin S. (Ed.) Aggregate Exposure a Report to EPA/ ILSI Workshop. ILSI Press, Washington, DC, 1998.
NCEH. Second national report on human exposure to environmental chemicals. Department of Health and Human Services Centers for Disease Control and Prevention, NCEH Pub. No. 02-0716, Revised, March 2003.
Novigen. Calendex™: Calendar-based dietary & non-dietary aggregate and cumulative exposure software system. Presented to FIFRA Scientific Advisory Panel (SAP) Arlington, VA, 27 September 2000.
Ott W. Computer simulation of human air pollution exposures to carbon monoxide. Paper 81-57.6. Paper presented at 74th Annual Meeting of the Air Pollution Control Association, Philadelphia, PA, 1981.
Price P.S., Chaisson C.F., Mathis E.T., and Tedder D.R. Cumulative risk assessment for organophosphorus pesticides using LifeLine™ version 2.0. Performed for the US EPA Office of Pesticide Programs by The LifeLine Group, Inc., 20 September 2002.

Price P.S., Curry C.L., Goodrum P.E., Gray M.N., McCrodden J.I., Harrington N.W., Carlson-Lynch H., and Keenan R.E. Monte Carlo modelling of time dependent exposures using a microexposure event approach. Risk Anal 1996a:16(3): 339–348.
Price P.S., Su S.H., Harrington J.R., and Keenan R.E. Uncertainty and variation in indirect exposure assessments: an analysis of exposure to tetrachlorodibenzo-p-dioxin from a beef consumption pathway. Risk Anal 1996b: 16(2): 263–277.
Rosenbaum A.S., Cohen J.P., and Kavoosi F. Update and refinement of an indoor exposure assessment methodology contract 98-327. Final Report. Prepared for California Air Resources Board, ICF Consulting, 2002.
Sielken R.L., and Holden L.R. Alpha CARES, population generator. American Crop Protection Association, 11 March 2001. The LifeLine Group. Technical manual for lifeline version 2.0. Software for Modelling Aggregate and Cumulative Exposures to Pesticides and Chemicals 2002.
US EPA. National home and garden pesticide use survey. Prepared by the Research Triangle Institute for the Office of Pesticides and Toxic Substances, Biological and Economic Analysis Branch 1992.
Zartarian V.G., Özkaynak H., Burke J.M., Zufall M.J., Rigas M.L., and Furtaw Jr. E.J. A modelling framework for estimating children's residential exposure and dose to chlorpyrifos via dermal residue contact and nondietary ingestion. Environ Health Perspect 2000: 108(6): 505–514.

Chapter 4

Synergy: A Risk Management Perspective

Paul S. Price

In The Principles and Practice of Mixtures Toxicology. Wiley- Verlag GmbH & Co. KGaA.. Ed. Moiz Mumtaz, 2010 Reproduced with permission.

Abstract

This chapter presents an assessment of current risk management practices for synergy. This approach is the reverse of the approach used in most discussions of synergy that focuses on the implications of synergy for risk management. In this chapter, we ask .What characteristics would synergistic effects need to have to cause the current methods for assessing chronic human noncancer effects to underestimate the toxicity of mixtures?. To answer this question, we review how synergy could affect the toxicity of a mixture and how noncancer risks of mixtures are determined and managed. These findings characteristics are used to investigate the relevance of specific findings of synergy and suggest directions for future research in this area. The chapter provides graphic and quantitative approaches tools for relating research findings of synergy to risk management decisions. An illustration of the application of these tools is provided. On the basis of the data available, it appears that many examples of synergy in the literature have little relevance to chronic noncancer risk management decisions and that existing approaches for risk management of mixture exposures that do not consider synergistic effects are protective.

Keywords: complex mixtures; critical effect; reference dose; relative potency factor; risk management; chronic; sensitive human NOAEL; interaction threshold.

Introduction

Any discussion of the risks from exposure to mixtures quickly comes round to the concept of synergy and the role that synergy plays in the toxicity of mixtures. All too often, at this point, the conversation ceases to be hopeful and the tone becomes one of quiet frustration [1]. There is a recitation of examples where synergy has been shown to occur and then a litany of reasons why it is difficult to address the issue. One might hear statements similar to the following: “Well the currently available in vivo tools are not amenable to investigating synergy,” “There are an infinite number of combinations we can’t test them all,” “We acknowledge the potential for synergy, but do not address it quantitatively,” and “There are no agreed upon methodologies for accounting for synergy.” At the end, one is left with the impression that whether or not synergistic effects actually pose a risk to public health, the understanding of the risks posed by synergy is still a work in progress.

This chapter investigates the issue of synergy using an approach that has been touched upon in prior publications [2], but has not been fully explored. Instead of asking how risk management can account for the occurrence of synergy, this

chapter asks what characteristics would be required in order for synergy cause risk management processes (that do not account for synergy), to underestimate the risks posed by mixtures of chemicals? This chapter begins with a review of synergy and the current practice of risk management of chemical mixtures. The estimates of the safe doses of mixtures and their components for test animals and humans permitted under current models of mixture toxicity are examined and the implications of the models for exposure to components of the mixture are discussed. A graphic approach is then presented for integrating findings of synergy into the risk management frameworks established by current models of mixture toxicity. An illustration of the approach is provided. Finally, we review the current literature on findings for low-dose interactions and recommendations for extrapolation of synergy observed at high doses to lower doses.

This chapter focuses on risk management of chronic noncancer effects in humans from exposure to discrete mixtures. Elevated toxicity due to synergistic interactions is an issue that also needs to be considered for acute noncancer and for cancer end points and for effects on environmental receptors (algae, fish, birds, etc.); however, these issues are not addressed in this chapter. Exposure to mixtures occurs in a variety of ways, such as exposures to discrete mixtures, cumulative exposures from multiple sources, co-exposure to parent compounds and metabolites, and so on. This chapter focuses on exposure to discrete mixtures; however the conclusions in this chapter may be relevant to other types of mixtures.

Synergy

The term synergy has been applied to a number of different phenomena [2, 3]. In this chapter, synergy is defined as a supra-additive dose response as a result of concurrent exposure to two or more chemicals [4]. Synergy has been observed to occur in a number of instances [5] and there are a number of mechanisms by which synergy occurs [5–7].

Synergy poses a number of challenges to assessing risks from mixtures. It appears as an emergent property of mixtures and is difficult to predict from the results of the limited in vivo studies that are typically available for chemicals [1]. The concern is that like a “wild card” synergy could potentially turn any combination of low-toxicity components into a toxic mixture of concern. Because synergy can elevate toxicity and is not readily predictable, it hangs like a cloud over mixture risk assessments.
The occurrence of synergy and other interactions is favoured by the laws of mathematics. Exposure to a mixture of two compounds (A and B) produces one

opportunity for synergy since there is one pair of chemicals (AB). Mixtures of three compounds (A, B, and C) have three opportunities for synergy since there are three unique combinations (AB, BC, and CA). For a mixture of 30 compounds there are 435 pairs and for a mixture of 100 compounds, 4950 pairs. By this argument, if synergy occurs in only one pair of chemicals in 10 000, then at least one instance of synergy would occur in half the mixtures containing 100 compounds.

Weighing against this prediction of enhanced toxicity in complex mixtures is the observation that most of the objects and media we encounter in life and all foods we consume are complex mixtures containing hundreds or thousands of compounds. In the case of food, many of these compounds are known to have considerable toxicity [8, 9].A full discussion of risk and benefits of the complex mixtures that form our foods is beyond the scope of this chapter, but it is clear that synergy must be either extremely rare or not result in significant increases in toxicity in foods that humans consume (see chapter 23 for additional discussion of mixtures in our diets).
Strategies to account for the occurrence of synergy in mixtures include:

- Performing testing of whole mixtures;
- Incorporating dose-response data from the joint testing of binary components; or
- Modelling the mechanism of dose interaction

The potential for synergy to affect a mixture's toxicity is most directly addressed by testing the toxicity of the entire mixture. This approach fully captures the interactions between components of the mixture. Testing individual mixtures; however is generally not feasible because of the vast number of mixtures and the expense of performing in vivo testing. This may change in the future with the rise of in vitro approaches to toxicity testing [7] (also see chapter 7).

The second option is to evaluate the interactions between individual components in mixtures and use these data to predict the behaviour of the entire mixtures. While synergy has been reported to occur in combinations of three or more compounds, characterizing the mechanisms of synergy has often been based on understanding the interaction between pairs of compounds [10–13]. The interactions in more complex mixtures are characterized by building on the understanding of binary interactions. Techniques to use data on binary mixtures include both PBPK-based approaches [10] and conceptual approaches (weight of evidence) [14–16] (see chapter 5 for additional discussion on this approach).

Finally, if the mechanism of action of each component in the mixture for the critical effect is known, then mechanistic models of the mixtures can be developed [7, 17]. All of these approaches require compound-specific data that may not be available for every component of mixtures of interest.

Risk Management and Synergy

Risk management of chemicals has been divided into two basic approaches. One approach is to assume no threshold and use a response addition model. This approach has been applied to cancer and genotoxic effects [15]. For other effects, the focus of risk management has been on estimating doses of chemicals that are unlikely to cause adverse effects in sensitive humans [18]. Under this approach, a battery of *in vivo* toxicity tests is used to identify a chemical's critical effect. The critical effect is the effect that occurs at the lowest dose as a result of either chronic or acute exposure. The dose response for this effect is used to establish a point of departure (POD). The POD could be a lowest observed effect level (LOAEL), a no-observed adverse effect level (NOAEL), no-observed effect level (NOEL), or benchmark dose (EPA noncancer policy [19]). The POD is then used to estimate a dose that is protective of sensitive individuals using a series of adjustment factors. These adjustment factors and POD produce conservative estimates of safe doses that reflect the toxicity of the chemicals and the quality of the toxicity data available for a particular chemical [20]. These estimates of safe doses have been given a variety of different names by different standard setting organizations including reference dose (RfD), tolerable daily intake, allowable daily intake, or the derived no-adverse effect level

Setting safe levels of a chronic exposure to a mixture is a two-step process. The first step is to develop an estimate of the toxicity of the mixture, the POD, based on in vivo study data of the mixture's components, the mixture itself, or the related mixtures [15]. The second step is the extrapolation of mixture's toxicity data in the test animal to an estimate of the safe dose for sensitive humans.

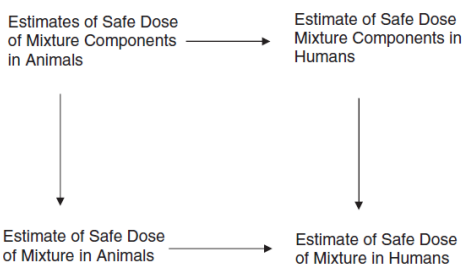

Figure 15.1 Two approaches for setting safe doses for mixtures

Interestingly, different mixture risk assessments have performed these two steps in different orders, Figure 15.1. For example, the toxicity of exposure to mixtures of organophosphorous pesticides in diet performed by EPA under the FQPA used relative potency factors (RPF) to estimate the animal toxicity of the mixture and then extrapolated the estimates to a safe dose for sensitive humans [21]. In contrast, the hazard index (HI) method for evaluating mixtures first converts the animal data on the components of the mixture to estimates of safe dose in humans (RfDs) and then estimates the safe dose of the mixture for humans [15].

Not all findings of synergy have a direct impact on the risk management of mixtures. Figure 15.2 presents results from two hypothetical examples of experimental data demonstrating synergy in mixtures containing two compounds A and B. In both examples, a dose of B (that does not cause an adverse effect) coadministered with a range of doses of A increases the response to A. In Example 1, B changes the threshold of A. This type of synergy is very relevant since it changes the POD of the mixture. Example 2 shows that B increases the response rate of A above the threshold but does not change A.s threshold. This type of synergy does not affect the current system of noncancer risk assessment since the threshold dose does not change.

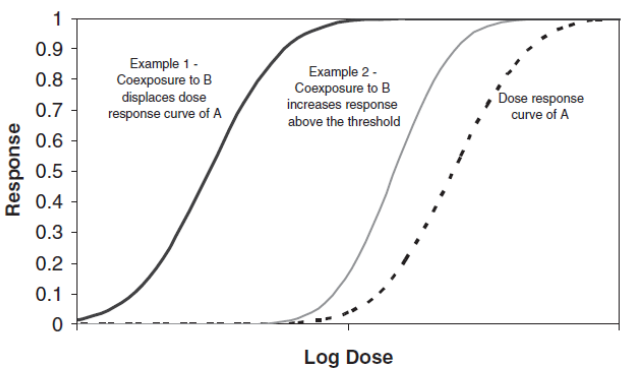

Figure 15.2 Examples of synergy that changes risk management decisions for mixture and synergy that has minimal impact

For this reason, findings about synergy that occur when one or both chemicals are below the NOAELs of the individual chemicals should receive additional attention. Such findings demonstrated that synergy both occurs and changes the mixture's threshold dose.

Mechanisms of chemical interaction can provide plausible justification for both types of synergy. If B facilitates the uptake of A, then it would shift the entire curve by increasing the effective dose at all dose levels of A. If B inhibits the recovery from the effects of A, then it would change the slope of the response but not the threshold dose.

There are also risk based issues why certain findings of synergy may not be relevant to the management of chronic effects. Demonstrating synergy is a resource-intensive process since testing of both the mixture and the mixture components is required. As a result, many studies of mixtures rely on acute and subchronic end points [22–24]. The organ or mechanism for the end points measured in the studies may not be the same as the critical end point used to set the chronic RfDs. If the end point is not related, then a finding of synergy may be of little importance for the management of the risk of chronic effects.

More importantly, acute end points generally require much higher doses than chronic end points (see Section 15.6). At these higher doses, the chemical components of the mixture cause a number of effects that are not adverse over the duration of the acute tests and appear as NOAELs. These effects are known to occur because over longer durations of exposure they lead to chronic toxicity. These effects can form the bases for observations of synergy when one or both chemicals may be at or below the acute NOAEL. This suggests that findings of synergy involving doses below an acute NOAEL would not necessarily imply that synergy would occur below chronic NOAELs, and again may not be relevant to setting chronic standards.

Models of Mixture Toxicity

A number of recent publications review models that can be used to estimate the safe doses of mixtures based on toxicity data on mixture components [6, 15, 25–27]. In the case of chronic noncancer effects where no data on interactions are available, the toxicity of mixtures is addressed using additive or independence models. The additive model has been described as being neutral with respect to synergy [15]. This model assumes that the components will neither suppress nor enhance each other's responses or that any deviations from additivity will cancel each other out. Neither the additive nor the independence models of mixture toxicity, however, directly account for the possibility that synergy will increase a mixture's toxicity.

Additive Models

Additive models of mixture toxicity assume that each chemical produces the same effects but at different doses. Adjusting for these differences using relative potency factors (RPFs) yields equivalent doses that can be summed to determine the mixture's response [6] (This approach is also discussed in chapters 3, 4, 10 and 18.)

Under additive models, the safe dose of a mixture can be determined from the prediction of the composition of the mixture and information on the toxicity of each component. The safety of mixtures is often expressed in terms of the hazard index (HI). Intakes of mixtures that result in HI values of less than 1 are considered to be safe. (In this approach, the RPF for a chemical is the inverse of each chemical's RfD). The value of the HI from exposure to one or more sources of multiple chemicals is given by Eq. (15.1).

$$\mathrm{HI} = \sum_{i}^{i} \frac{D_i}{\mathrm{RfD}_i}, \tag{15.1}$$

where D_i is the dose of the *i*th component of the mixture (mg/kg/day) and RfD_i is the reference dose of the *i*th component (mg/kg/day). The ratio of the dose to the RfD has traditionally been called the hazard quotient (HQ) [15].

Additive models of mixture risk can be used to derive an estimate of the safe dose of any discrete mixture where the composition of the mixture is known and RfDs are available for each component of the mixture. This mixture RfD is calculated using Eq. (15.2) [15]

$$\text{Mixture RfD} = \frac{1}{\sum_{i}^{i} \frac{F_i}{\mathrm{RfD}_i}}, \tag{15.2}$$

where F_i is the fraction of the mass of the mixture for the *i*th component of the mixture.

An alternative additive model that has been used for pesticides is based on a benchmark chemical and RPFs. Ratios of consistent measurements of response such as ED10 have been used to set the RPF values. Under this approach, the toxicity of a mixture is

$$\text{Mixture RfD} = \frac{\mathrm{RfD_B}}{\sum_{i}^{i} \frac{F_i}{\mathrm{RPF}_i}}, \tag{15.3}$$

where RfD_B is the reference dose for the benchmark chemical, and RPF_i is the RPF for the *i*th chemical. Other variations on the additive models also exist [15, 26].

Independence Models

The independence models make the explicit assumption that none of the mixture's components interact. While there is no interaction, the responses from each component are still added [6]. This response addition could influence the determination of the POD if the POD is based on a benchmark dose [15]. (chapter 10 provides additional discussion on the independence model.)

Placing doses used in studies demonstrating synergy into a risk management framework

Setting a safe level for mixtures using the independence assumption involves setting a separate assessment for each mixture component. In these component-specific estimates, the safe dose of the mixture is determined on the basis of the toxicity of the one component and assuming that the other contaminants merely dilute the component. A dose of a mixture that would allow a safe dose of the *i*th component of the mixture where the mass fraction of the component in the mixture (F_i) is given by

$$\text{Safe dose from } i\text{th component} = \frac{\text{RfD}_i}{F_i}. \tag{15.4}$$

The mixture reference dose determined by an independence model would be the minimum value of the safe dose from the *i*th component for all of the mixture's components.

$$\text{Mixture RfD} = \min\left(\frac{\text{RfD}_i}{F_i}\right) \tag{15.5}$$

Exposure to the Components Permitted Under Additive and Independence Models

The dose of each chemical component of a mixture that will occur when an individual receives a safe dose of a mixture (e.g., a dose less than the safe dose estimated by an additive or independence model) is a function of the dose of the mixture and the values of F_i for the individual components. The doses of the components permitted under the additive and independence models are

$$\text{Dose}_i = \frac{F_i}{\sum_i \frac{F_i}{\text{RfD}_i}}, \tag{15.6}$$

$$\text{Dose}_i = F_i \times \min\left(\frac{\text{RfD}_i}{F_i}\right). \tag{15.7}$$

Under the additive model, individuals exposed to the maximum dose of the mixture will not receive a dose of any mixture component greater than the safe dose (RfD) of the component. Under the independence model, individuals exposed to the maximum dose of the mixture will receive a dose of one mixture component that is equal to the RfD of that component. Doses of remaining components will be at or below their respective RfDs.

Placing Doses Used in Studies Demonstrating Synergy into a Risk Management Framework

A graphic approach is used to place the doses of mixtures used in synergy studies and the doses allowed under different risk models into a common framework. This approach builds on the isobologram format used in many mixtures studies. The presentation of this approach takes into account the two-step process for setting safe levels of mixtures for sensitive humans (see Figure 15.1). The first step of the process is to characterize the doses permitted to *test animals* that would be allowed under additive and independent models using the Eqs. (15.7) and (15.8). The second step is to examine how the RfD-setting process affects the doses permitted for humans under the use of RfD-based models of mixture risks. The first step is presented in this section and the second step in Section 15.7.

We begin the presentation of the approach with mixtures containing two compounds then expands the approach to more complex mixtures. Figure 15.3 presents the range of possible doses of two chemicals A and B (D_A and D_B) normalized to the chemicals' chronic NOAELs. The chronic NOAELs are defined in terms of the absence of all adverse effects of the chemical in a test animal. The normalized doses appear as two axes in the graph. The doses of A and B received as a result of exposure to a specific mixture appear as a point on this graph. The single point in Figure 15.3 indicates a study where an exposure to a mixture resulted in a dose that is three times the chronic NOAELs of chemicals A and B.

The graph can be divided into different regions. Region 1 consists of the combinations of doses where both the doses of both compounds exceed their respective chronic NOAELs. Region 2 consists of the combinations of doses where one of the chemical's doses exceeds the chemical's NOAEL. Under risk management

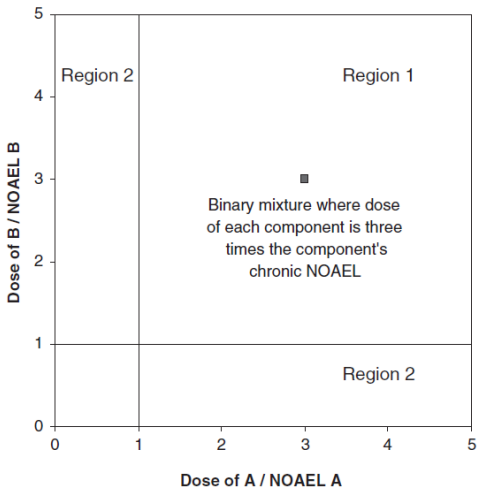

Figure 15.3 Plotting the normalized doses and identifying regions of unacceptable chronic doses of binary mixtures

decision making, chronic doses of mixtures that fall into regions 1 and 2 are not permitted, not because of concerns for synergetic effects but because of the toxicity of either one or both mixture components.

In the case of a mixture of chemicals A and B, the maximum doses of chemicals A and B that are permitted from safe level of exposure to the mixture for the test animals are limited. For the additive model these Levels are given by Eq. (15.8).

$$1 \leq \frac{D_A}{\text{NOAEL}_A} + \frac{D_B}{\text{NOAEL}_B} \qquad (15.8)$$

Under independence models of mixture risk, Eq. (15.5), the permitted doses are also restricted to doses below the NOAELs; however, the permitted doses are independent of the concentration of the other component. The doses of the components are given by Eq. (13.9).

$$1 \leq \frac{D_A}{\text{NOAEL}} \quad \text{and} \quad 1 \leq \frac{D_B}{\text{NOAEL}} \qquad (15.9)$$

The independence model (13.9) allows dose combinations that fill the entire remaining region in Figure 15.4. The additive model (13.8) allows the dose combinations that fill a triangular area close to the origin. Region 3 is defined as dose combinations that are not allowed under an additive model but are allowed

under an independence model. Region 4 is defined as doses that are allowed under both models.

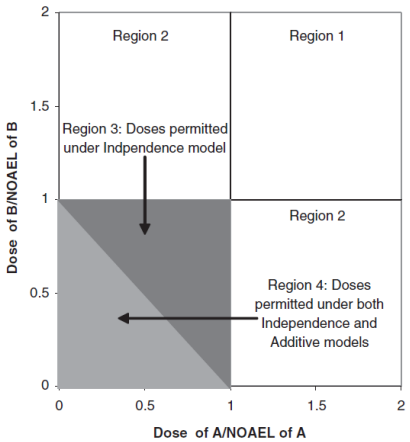

Figure 15.4 Identifying regions of acceptable chronic does under additive and independence models of mixture toxicity

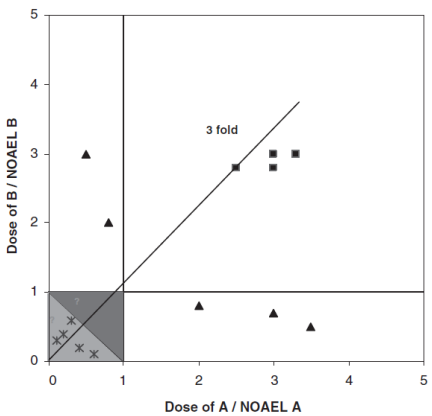

Figure 15.5 Placing doses from synergy studies into the four regions and describing extrapolation of synergy findings from regions 3 and 4

With this framework, it is possible to place data on synergy in binary mixtures into a risk management context. The doses of the binary components in studies that demonstrate synergy can be assigned a location in one of these four regions (Figure 15.5). Studies that have demonstrated synergy for dose combinations that fall in regions 1 and 2 are not directly relevant to setting safe doses of mixtures for chronic exposures. The additive and independence mixture models already have determined that such mixtures are unsafe because of the concern for the toxicity

of the mixture components. Thus the finding of synergy does not change the risk management decision for the mixture. Observations of synergy in mixtures that fall into region 3 are relevant for setting safe doses of mixtures under an independent model since such doses would be permitted under such a model. The doses that fall into region 4 would be relevant for setting safe doses under either independence or additive models.

Data on synergy from dose combinations that fall into regions 1 and 2 could still suggest an indirect concern if the available data imply that the synergy observed at higher doses persists at lower doses. (These lower doses appear as a ray in Figure 15.5.) Thus, if data suggest that a threefold increase in response above additivity observed at doses of A and B that are three times the test animal's chronic NOAELs, and the data suggest that some portion of the increase also occurs at doses at the animal's NOAELs, then the finding will be relevant for independence models of mixture toxicity. If the data imply that an increase also occurs at doses less than 50%of the animal's chronic NOAELs (region 4), it also would be relevant to doses permitted under additive models.

Extending the Approach to Mixtures of Three or More Chemicals

The above approach for displaying the doses of mixture components can be extended to more complex mixtures. As discussed above, mixture of three or more compounds will have three or more unique pairs of chemicals. Each pair from a mixture that has been shown to cause synergistic effects can be plotted in one graph. For consistency sake, the larger of the two normalized doses is plotted on the x-axis and the smaller of the two normalized doses are plotted on the y-axis. This allows the display of how each pair of the chemicals in a mixture compares to the chronic NOAELS of the chemicals.

An illustration of how a more complex mixture can be plotted is provided in Figure 15.6. This figure presents the normalized doses of components in a 10 mg/kg dose of a mixture of five organophosphorous pesticides tested by Moser *et al*. [23].

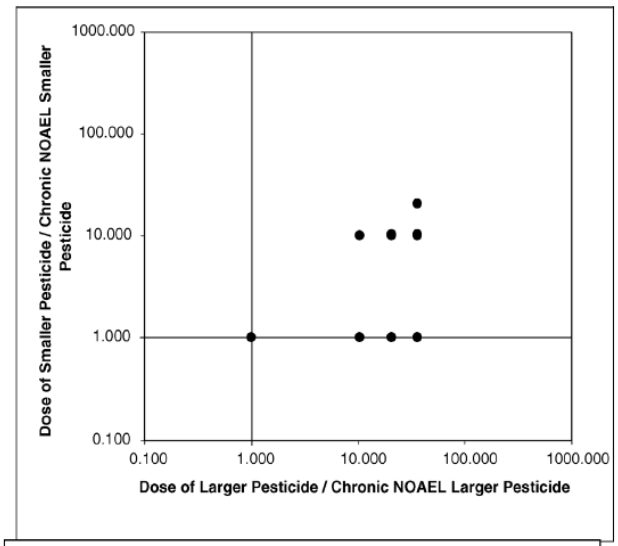

Figure 15.5 Placing doses from studies of synergy in more complex mixtures.

Since there are 5 pesticides, there are 10 unique pairs of pesticides that appear as 10 points on the graph. All doses of the mixture components in the 10/mg dose groups are at or above the no-observed effect level used to set the chronic standards of the components. The data point for the largest pair of the pesticides (based on a fraction in the mixture and toxicity) occurs between Malathion (36 times it's (No Observed Effect Level (NOEL)) and Dimethoate (20 times its NOEL).

Using the Graphic Framework to Place Data on Synergy into a Risk Management Context

The Moser *et al.* study [23] investigated a mixture of five pesticides determined to co-occur at specific relative ratios in U.S. diets. The mixture was studied in a ray design where the composition of the mixture is held constant but the doses of the mixture are varied. The individual pesticides were studied separately and an additive model of the mixture's toxicity was developed and used to predict the response of the mixture at the different doses. The actual responses were higher than those predicted by the additive model indicating a synergic response. Table 15.1 presents the fraction of each pesticide in the mixture, the doses administered in the three lowest doses in the study (10, 20, and 40 mg/kg of the mixture), the degree of synergy (ratio of the value from a response curve fitted to the observed data to the value from the response curve predicted by the additive model), and

the NOEL used in setting the compounds' chronic standards. The magnitude of synergy at the lowest dose estimated was 2.5 times higher than the additive model predicted and increased to 20 times higher at 40 mg/kg.

Because the composition of the mixture is known and the NOELs for each compound are available, it is possible to estimate the maximum doses of the mixture and its components that would be allowed under additive and independence models (Table 15.2). Figure 15.7 presents the data points for top pair of pesticides in the three test doses and in the doses permitted under additive and independence models. In order to display the results of the study, the dose/NOEL ratios are presented on a log scale. In addition, the size of the synergistic factor associated with each of the doses is given above the data point for each dose group. Note, in order to easily place the data on the figure, the ratios of dose to NOAEL are placed on a log scale. As a result, the boundary between regions 3 and 4 now appears as a curve.

Table 15.1 Data on components of organophosphorous pesticide mixture

Mixture	Chlorpyrifos	Acephate	Diazinon	Dimethoate	Malathion
NOAEL[a)]	0.03	0.04	0.02	0.05	0.23
Fraction of mixture[b)]	0.031	0.04	0.002	0.102	0.825

a) As reported for the pesticide's RfDs [46] or in the Reregistration Decision [47].
b) Moser *et al.* [23].

nd synergy measurements for mixtures of organophosphorous pesticide mixtures.

Chlorpyrifos	Acephate	Diazinon	Dimethoate	Malathion	Total mixture	Synergy observed[a)]
e lowest dose groups (mg/kg/day)						
1.2	1.6	0.080	4.1	33	40	20
0.62	0.80	0.040	2.0	17	20	5.6
0.31	0.40	0.020	1.0	8.3	10	2.5
nder additive and independence models (mg/kg/day)						
0.0086	0.011	0.00 056	0.028	0.23	0.28	—
0.0040	0.0052	0.00 026	0.013	0.11	0.13	—

as the rate of response observed divided by the rate predicted by the author's additive model at each of the dose levels as presented in Figure 6 of Ref. [23].

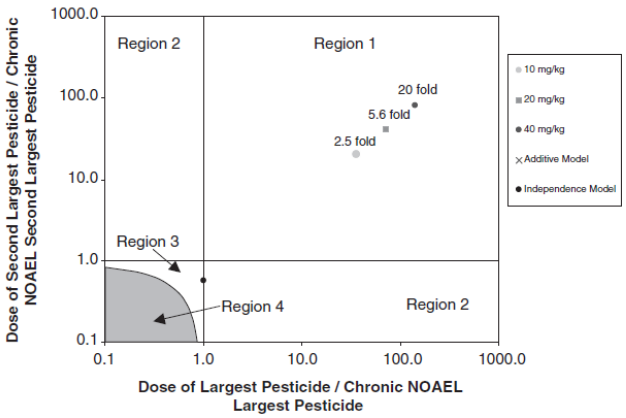

Figure 15.7 Plot of the highest pair of components of the 10, 20, 40 mg/kg/day dose groups from Moser *et al.* [23] and the corresponding maximum doses permitted under independence and additive models.

As Table 15.2 and Figure 15.7 show, the smallest of the administered doses, 10 mg/ kg, in the Moser *et al*. study is 78 times larger than the doses that would have been permitted under an additive model and 36 times the doses that would be allowed under an independence model of animal toxicity. This finding is not surprising since a single dose was used in the study; however this indicates that the finding of synergy in this study falls into region 1 and is not directly relevant to the management of mixtures of organophosphorous pesticides.

The data from the study also provide insight into whether the study's findings could provide indirect evidence of synergy at lower doses. The level of the synergy observed was highly dose dependent. The synergy declined at lower doses dropping from 20- to 2.5-fold over a 4-fold change in dose. Given this finding, it is unlikely that synergy will persist over an additional 36–78-fold drop in dose. Thus, while the finding of synergy in this study may be useful in understanding the mechanism by which various organophosphorous pesticides interact at high doses, it does not provide compelling evidence that an additive model will underestimate chronic risks from the organophosphorous pesticides.

This illustration also demonstrates that chemicals that have lower toxicity and occur at lower concentrations will be restricted to smaller doses under both models of mixture toxicity. As shown in Table 15.2, exposure to one of the five pesticides was limited to 1/80 of the chemical's NOEL and two others to 1/8 of

their NOELs. The impact of this is a reduction of the number of pairs of chemicals where synergy is likely to occur. While there are ten unique pairs of pesticides in the mixture, there is only one pair of pesticides, Malathion and Dimethoate, where the doses of both chemicals are greater than 25% of the chronic NOAELs.

Doses of Mixture Components Permitted Under Current Models of Mixture Risks for Humans

Discussions in Sections 15.5 through 15.7 only address the issue of synergy in determining safe doses of mixtures in animals. The ultimate goal of mixture risk assessment is to identify safe doses that protect sensitive humans. As a result, assessments of the risks posed to humans must address from animal data to a dose that is protective of a sensitive individual. This extrapolation is performed using a series of uncertainty factors that convert the POD to RfDs.

Recent Findings on the Relationship between Chronic Toxicity in Sensitive Humans and Reference Doses

In the case of a two chemical mixture, the doses of chemicals A and B that are permitted for humans under additive models are given by Eq. (15.10) and independence models by Eq. (15.11).

$$1 \leq \frac{D_A}{\mathrm{RfD}_A} + \frac{D_B}{\mathrm{RfD}_B} \tag{15.10}$$

$$1 \leq \frac{D_A}{\mathrm{RfD}_A} \quad \text{and} \quad 1 \leq \frac{D_B}{\mathrm{RfD}_B} \tag{15.11}$$

The relationship between the RfD and the chronic sensitive human NOAEL (CSHN) has been the subject of considerable debate. Hertzberg and Teuschler [16] acknowledged the issue but concluded that the RfD could be considered to be "equally uncertain and equally biased" estimates of the sensitive human NOAEL. Support for this position is implicit in the EPA definition of the RfD.

> An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. (EPA [18])

This finding implies that the same regions can be established for sensitive humans using RfDs instead of the chronic animal NOAEL (Figure 15.8).

This position has been challenged by the work of a number of researchers in the United States and European Union who have investigated the uncertainty in the

CSHN and its relationship with the RfD [28–34]. These studies consistently find that RfDs are in fact a very biased estimate of the chronic sensitive human NOAEL (CSHN). The majority of the published RfDs (>90%) established using the current system of safety factors have values that are at or below the chemical's actual CSHN. The typical RfD set with two 10-fold safety factors is likely to be an order of magnitude below the actual CSHN for the chemical. When additional safety factors are used, the typical value of the RfD is even lower compared to the chemical's actual CSHN.

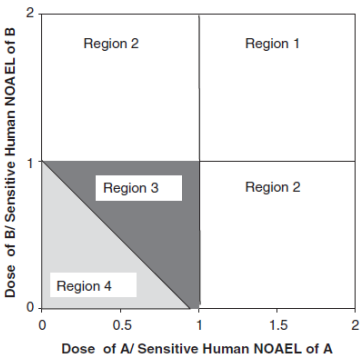

Figure 15.8 Regions 1-4 for sensitive humans based on the assumption that the RfD is an accurate and unbiased estimate of the chronic NOAEL for the sensitive human

The impact of this systematic underestimation of the CSHN by the RfD results in a reduction in the fraction of the sensitive human NOAEL that is permitted in the doses of the components in the mixture risk models. When additive models or independence models are used with RfDs that there are lower than the CSHN the areas covered by regions 3 and 4 are reduced. Figure 15.9 presents the size of the regions when the RfD of mixtures A and B are both 25%[8]) of the CSHN.

In order for synergy to be relevant under this situation it must occur at doses that are a small fraction of the CSHN.

Impact of the Current System of Safety Factors on the Moser *et al.* [23] Data

The chronic standards for the organophosphorous pesticides are established with various numbers of safety factors and the total safety factors applied to the

[8] The value of 25% was selected as a reasonable amount of over estimation (4 fold). The cited references suggest that this degree of over estimation of toxicity would occur for the majority of chemicals established with the current system of uncertainty factors.

pesticides. NOAELs range from 10 to 3000. The impact of the use of the safety factors in extrapolating from animal to sensitive humans can be shown in Table 15.3.

The permitted doses of each pesticide for the test animals can be calculated using Eqs. (15.6) and (15.7). Table 15.3 presents these doses normalized to the animal NOAELs. The results show that under the additive model the compound with the highest fraction of its NOAEL is Malathion (0.46). Under the independence model, the highest fraction is again Malathion (1.0). If we assume that the RfD is 25% of the CSHN the equivalent values for humans would be 0.12 and 0.25.

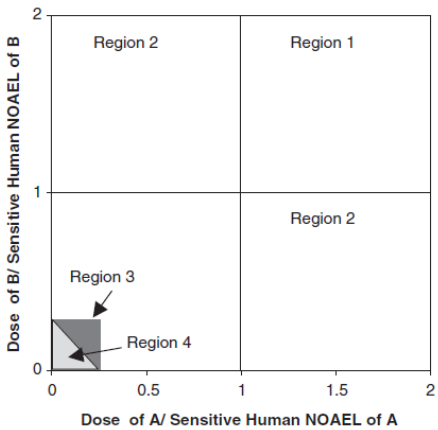

Figure 15.9 Regions 1-4 for sensitive humans based on the assumption that the RfD is an overestimate of the chronic NOAEL for the sensitive human by a factor of 4

These findings indicate that it is likely that the additive and independence model will keep human exposure to small fractions of the CSHN. This finding has profound implications for synergy. It requires that synergy must affect humans at doses that are significantly less than the CSHN. In the case of the additive model, the pair of components Dimethoate and Malathion have the highest ratios (0.088 and 0.15). All other pairs of pesticides involve exposures that occur at even smaller fractions of the CSHN.

Table 15.3 Doses to animals and humans permitted under additive and independence models

	Maximum dose permitted to animals/animal NOEAL		Maximum dose permitted to humans/actual chronic sensitive human NOAEL	
	Additive model	Independence model	Additive model	Independence model
Chlorpyrifos	0.13	0.29	0.034	0.072
Acephate	0.13	0.28	0.033	0.072
Diazinon	0.013	0.028	0.0032	0.0070
Dimethoate	0.26	0.57	0.066	0.14
Malathion	0.46	1.0	0.12	0.25

Relationship between Toxicity and Synergistic Potential

The above discussion has demonstrated that in order for synergy to be relevant to the characterization of chronic effects from discrete mixtures, it must occur at doses well below CSHN. The existence of synergy at levels well below NOAELs is not a new question [2]. This section presents a brief summary of the literature on this topic.

What are "Low Doses?"

Publications on chemical interactions have defined "low doses" in a number of ways. These multiple definitions have led to a great confusion in the relevance of findings to the risk assessment process. The term "low dose" has been used in multiple ways.

1) Doses causing frank effects but below acutely toxic doses (LD_{50}) in single dose studies. Synergy has often been reported to occur on the basis of observations in acute toxicity (single doses) of mixtures.
2) Repeated doses but over a small number of days (subacute studies) that cause frank effects but not lethality. In many instances, the end point where synergy is observed may not be a measure of the critical effect for chronic durations.
3) Doses near or below the NOAEL seen in chronic studies [36].
4) Doses below the chronic health standards [2].

As discussed above, from a risk management perspective, the third use is the correct one. As demonstrated in Section 15.6, Eqs. (15.4) and (15.6) provide a direct method to determine the maximum chronic doses of a mixture, and its components, that are permitted under additive and independence models. These estimates can be used to check the dose levels of any mixture where synergy has been identified.

Does Synergy Occur at Low Doses?

In the 1980s, TNO began a program to look at the occurrence of chemical interaction at low doses [37]. The results of this work were published in a series of papers [38–43] that form the largest set of data mixture interaction for subchronic effects. These papers are the largest group of studies that look at doses closest to the chronic NOAELs of chemicals. The general conclusion reached from this body of work was that at these doses additive models appeared to be appropriate for groups of chemicals that have a common end point. Mixtures of chemicals that operated by different mechanisms and affect different organs were best predicted by an independence model [37]. Little or no evidence of synergy was observed. Konemann and Pieters [2] argue similar findings based on data from aquatic toxicology.

These findings are based on a relatively small number of studies (<20). As a result, there is a need for additional research to determine whether these findings will be sustained by additional testing and the authors acknowledged that there might be exceptions to these findings [41].

Can Findings of Synergy at High Doses be used to characterize the Potential for Synergy at Low Doses?

The literature presents a surprisingly consistent answer to this question. Findings on interactions at high doses do not predict the existence of interactions at lower doses [24]. Empirical findings from ray studies such as Gennings [22], Moser [23], and Crofton [35] have shown that synergy is a function of dose and that as doses decline the intensity of synergistic effects declines. Other studies have shown even more complex dose responses including changes from antagonism at high doses to synergy and additivity at lower doses [44]. Because of these observations, Groten [23], Konemann and Pieters [2], and Feron and Groten [36] have strongly argued that there is no evidence that synergy observed at high doses can be automatically applied to lower doses. Gennings *et al*. [45] has termed the dose dependent nature of synergy as .interaction threshold. (The reader is referred to the extensive discussion on the measurement of the interaction threshold in chapters 4 and 7.)

Konemann and Pieters further argue that the mechanisms by which synergy occurs imply that synergy is a dose-dependent phenomena. They state that there is “little doubt that combined dose action of compounds is a dose-dependent phenomenon. At low doses, “physiochemical interactions are of relatively low importance and toxicokinetic, and toxicodynamic may also be very rare” [2].

Finally, the doses of mixture components that occur as a result of exposure to discrete mixtures also favour dose dependency in synergistic effects. In discrete mixtures, the exposures to any two chemicals are fixed by their relative fractions in a mixture. As the dose of one chemical declines so will the second. Consider Example 1 from Figure 15.2 where a dose of chemical B enhanced the toxicity of chemical A at all doses of A. In the case of a discrete mixture, the dose of B declines with A. If the effect of B on A occurs by any mechanism that is dose dependent, then reducing the exposure to the mixture will result in a reduction in synergy because the dose of B declines, so does the dose of A.

Discussion

This chapter has outlined a series of arguments on the potential occurrence of synergy in humans exposed to the maximum doses permitted under different models currently used by risk assessors to manage exposure to mixtures. Current risk management practices limit chronic exposures to mixture components to levels that are below chronic NOAELs for the chemicals in test animals. In simple binary mixtures, the permitted exposures could approach the component chemical's NOAELs, especially when independence models are used. However, if additive models are used and if the mixtures include larger numbers of components, the mixtures' components will be restricted to doses much lower than the component's animal NOAELs. Current practices for extrapolating from animal data to sensitive humans have the net effect of further reducing the ratio of permitted doses to the mixture components' actual CSHNs. As seen in the Moser et al. example, only one of 10 possible pairs of chemicals had appreciable to both chemicals (Table 13.3). This pattern is expected to occur in all mixtures with 3 or more components.

A review of the literature suggests that synergy is least likely to occur at such low doses. Existing studies that have demonstrated synergy have been performed at higher doses (region 1). Extrapolation of synergistic effects at higher doses to lower doses does not appear to be justified on either an empirical or a theoretical basis. As a result, the existing empirical findings on synergy do not appear to provide evidence that the estimates of safe doses produced by additive and independence models in chronic noncancer risk assessments are not protective. The existing findings of synergy may be more relevant to the establishment of acute toxicity standards, but the assessment of such standards is beyond the scope of this chapter.

These findings present a conundrum for mixture toxicologists who are trying to empirically confirm the safety of the doses of mixtures predicted by the models. The costs of long-term animal studies are so large that it is impossible to investigate the occurrence of chemical interactions at doses below the chronic NOAELs used in assessing mixtures. Short-term studies require higher doses that fall in region 1 and thus are not directly relevant to noncancer assessments. Finally, the existing literature strongly warns against extrapolating findings of synergy from high doses to low doses.

The solution to this problem will not be found by continuing the current practice of testing mixtures at high doses. Rather, new techniques and short-term assays are required to detect and evaluate the low-level molecular and cellular changes that are precursors to chronic toxicity [7, 17]. Techniques, such as toxicogenomics, proteomics, and signalling pathway assessments could be used to investigate chemical interactions.

Summary and Conclusions

A complete review of the literature on synergy and a determination of the relevance of each published study for the assessment of the toxicity of mixtures are beyond the scope of a single book chapter. The key finding of this effort is that synergy is a phenomenon that occurs only at doses where one or both chemicals pose unacceptable risk to humans and thus has no direct impact on chronic risk management decisions. In this chapter, we have outlined an approach for determining the relevance of existing and future findings of synergy that can be applied to any discrete mixture where the mixture's composition is defined and the chronic toxicity of the components are known.

References

1 Borgert, C.J. (2004) Chemical mixtures: an unsolvable riddle? Hum. Ecol. Risk Assess., 10, 619–629.
2 Konemann, W.H. and Pieters, M.N. (1996) Confusion of concepts in mixture toxicology. Food Chem. Toxicol., 34, 1025–1031.
3 Groten, J.P. (2000) Mixtures and interactions. Food Chem. Toxicol., 38, S65–S71.
4 Gennings, C., Carter, W.H., Jr., Carchman, R.A., Teuschler, L.K., Simmons, J.E., and Carney, E.W. (2005) A unifying concept for assessing toxicological interactions: changes in slope. Toxicol. Sci., 88, 287–297.
5 Greco, W.R., Bravo, G., and Parsons, J.C. (1995) The search for synergy: a critical review from a response surface perspective. Pharmacol. Rev., 47, 331–385.
6 IGHRC (2009) Chemical Mixtures: A Framework for Assessing Risks.
7 Yang, R. (2004) Chemical mixture toxicity: from descriptive to mechanistic and going on to *in silico* toxicology. Environ. Tox. Pharmacol., 18, 65–81.
8 Culliney (1992) Pesticides and natural toxicants in foods. Agric. Ecosyst. Environ., 41, 297–320.

9 Mattsson, J.L. (2007) Mixtures in the real world: the importance of plant self-defense toxicants, mycotoxins, and the human diet. Toxicol. Appl. Pharmacol., 223, 125–132.
10 Haddad, S. and Krishnan, K. (1998) Physiological modelling of toxicokinetic interactions: implications for mixture risk assessment. Environ. Health Perspect., 106 (Suppl. 6), 1377–1384.
11 Haddad, S., Tardif, R., Charest-Tardif, G., and Krishnan, K. (1999) Physiological modelling of the toxicokinetic interactions in a quaternary mixture of aromatic hydrocarbons. Toxicol. Appl. Pharmacol., 161, 249-257.
12 Haddad, S., Charest-Tardif, G., Tardif, R., and Krishnan, K. (2000) Validation of a physiological modelling framework for simulating the toxicokinetics of chemicals in mixtures. Toxicol. Appl. Pharmacol., 167, 199–209.
13 Haddad, S., Charest-Tardif, G., and Krishnan, K. (2000) Physiologically based modelling of the maximal effect of metabolic interactions on the kinetics of components of complex chemical mixtures. J. Toxicol. Environ. Health A, 61, 209–223.
14 Mumtaz, M.M. and Durkin, P.R. (1992) A weight-of-evidence approach for assessing interactions in chemical mixtures. Toxicol. Ind. Health, 8, 377–406.
15 US EPA (2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. US Environmental Protection Agency, Office of Research and Development, Washington, DC.
16 Hertzberg, R.C. and Teuschler, L.K. (2002) Evaluating quantitative formulas for dose-response assessment of chemical mixtures. Environ. Health Perspect., 110 (Suppl. 6), 965–970.
17 Teuschler, L., Klaunig, J., Carney, E., Chambers, J., Conolly, R., Gennings, C., Giesy, J., Hertzberg, R., Klaassen, C., Kodell, R., Paustenbach, D., and Yang, R. (2002) Support of science-based decisions concerning the evaluation of the toxicology of mixtures: a new beginning. Regul. Toxicol. Pharmacol., 36, 34–39.
18 US EPA (2008) Reference Dose (RfD): Description and Use in Health Risk Assessments: Background Document 1A, December 1. http://www.epa.gov/IRIS/rfd.htm.
19 US EPA (2008) EPA Guidance for Noncancer Risk Assessments, March 3. http://www.epa.gov/ttn/atw/toxsource/noncarcinogens.html.
20 Dourson, M.L. (1994) Methodology for establishing oral reference doses (RfDs), in Risk Assessment of Essential Elements, ISLI Press.
21 US EPA (2002) Organophosphate Pesticides: Revised OP Cumulative Risk Assessment; Washington, DC.
22 Gennings, C., Carter, J.W.H., Casey, M., Moser, V., Carchman, R., and Simmons, J.E. (2004) Analysis of functional effects of a mixture of five pesticides using a ray design. Environ. Tox. Pharmacol., 18, 115–125.
23 Moser, V.C., Simmons, J.E., and Gennings, C. (2006) Neurotoxicological interactions of a five-pesticide mixture in preweanling rats. Toxicol. Sci., 92, 235–245.
24 Groten, J.P. (2000) Mixtures and interactions. Food Chem. Toxicol., 38, S65–S71
25 Agency for Toxic Substances and Disease Registry (ATSDR) (2002) Guidance Manual for the Assessment of Joint Toxic Actions of Chemical Mixtures. US Department of Health and Human Services, Public Health Service, Atlanta, GA.
26 Mumtaz, M.M., Poirier, K.A., and Colman, J.T. (1997) Risk assessment for chemical mixtures: fine-tuning the hazard index approach. J. Clean. Technol., Environ. Toxicol. Occup. Med., 6, 189–204.
27 Teuschler, L.K. (2007) Deciding which chemical mixtures risk assessment methods work best for what mixtures. Toxicol. Appl. Pharmacol., 223, 139–147.
28 Baird, S.J.S., Cohen, J.T., Graham, J.D., Shlyakter, A.I., and Evans, J.S. (1996) Noncancer risk assessment: a probabilistic alternative to current practice. Hum. Ecol. Risk Assess., 2, 79–102.
29 Swartout, J.C., Price, P.S., Dourson, M.L., Carlson-Lynch, H.L., and Keenan, R.E. (1998) A probabilistic framework for the reference dose (probabilistic RfD). Risk Anal., 18, 271–282.
30 Slob, W. and Pieters, M.N. (1998) A probabilistic approach for deriving acceptable human intake limits and human health risks from toxicological studies: general framework. Risk Anal., 18, 787–798.

31 Bosgra, S., Bos, PM., Vermeire, TG., Luit, RJ., Slob, W. (2005) Probabilistic risk characterization: an example with di(2-ethylhexyl) phthalate. Regul. Toxicol. Pharmacol., 43 (1): 104–113.
32 Gaylor, D.W. and Kodell, R.L. (2000) Percentiles of the product of uncertainty factors for establishing probabilistic reference doses. Risk Anal., 20, 245–250.
33 Kalberlah, F., Schneider, K., and Schuhmacher-Wolz, U. (2003) Uncertainty in toxicological risk assessment for non-carcinogenic health effects. Regul. Toxicol. Pharmacol., 37, 92–104.
34 Schneider, K., Oltmanns, J., and Hassauer, M. (2004) Allometric principles for interspecies extrapolation in toxicological risk assessment–empirical investigations. Regul. Toxicol. Pharmacol., 39, 334–347.
35 Crofton, K.M., Craft, E.S., Hedge, J.M., Gennings, C., Simmons, J.E., Carchman, R.A., Carter, W.H., Jr., and DeVito, M.J. (2005) Thyroid-hormone disrupting chemicals: evidence for dose-dependent additivity or synergism. Environ. Health Perspect., 113, 1549–1554.
36 Hendriksen, P.J., Freidig, A.P., Jonker, D., Thissen, U., Bogaards, J.J., 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.
37 Feron, V.J. and Groten, J.P. (2002) Toxicological evaluation of chemical mixtures. Food Chem. Toxicol., 40, 825–839.
38 Jonker, D., Woutersen, R.A., van Bladeren, P.J., Til, H.P., and Feron, V.J. (1990) 4-Week oral toxicity study of a combination of eight chemicals in rats: comparison with the toxicity of the individual compounds. Food Chem. Toxicol., 28, 623–631.
39 Jonker, D., Woutersen, R.A., van Bladeren, P.J., Til, H.P., and Feron, V.J. (1993) Subacute (4-week) oral toxicity of a combination of four nephrotoxins in rats: comparison with the toxicity of the individual compounds. Food Chem. Toxicol., 31, 125–136.
40 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.
41 Cassee, F.R., Groten, J.P., van Bladeren, P.J., and Feron, V.J. (1998) Toxicological evaluation and risk assessment of chemical mixtures. Crit. Rev. Toxicol., 28, 73–101.
42 Groten, J.P., Schoen, E.D., van Bladeren, P.J., Kuper, C.F., van Zorge, J.A., and Feron, V.J. (1997) Subacute toxicity of a mixture of nine chemicals in rats: detecting interactive effects with a fractionated two-level factorial design. Fundam. Appl. Toxicol., 36, 15–29.
43 Feron, V.J., Cassee, F.R., Groten, J.P., van Vliet, P.W., and van Zorge, J.A. (2001) International issues on human health effects of exposure to chemical mixtures. Conference on Application of Technology to Chemical Mixtures Research, Fort Collins, CO, January 9–11, 2001, Fort Collins, CO, Abstract S-5.
44 Gennings, C., Carter Jr., W.H., Campain, J.A., Bae, D., Yang, R.S.H. (2002). Statistical analysis of interactive cytotoxicity in human epidermal keratinocytes following exposure to a mixture of four metals. J. Agric. Biol. Environ. Stat. 7, 58–73.
45 Gennings, C., Carter, W.H., Carchman, R.A., DeVito, M.J., Simmons, J.E., and Crofton, K.M. (2007) The impact of exposure to a mixture of eighteen polyhalogenated aromatic hydrocarbons on thyroid function: estimation of an interaction threshold. JABES, 12(1), 96.
46 Integrated Risk Information System (IRIS). Accessed March 1, 2008.
47 US EPA (2006) Finalization of interim reregistration eligibility decisions (IREDs) and interim tolerance reassessment and risk management decisions (TREDs) for the organophosphate pesticides, and completion of the tolerance reassessment and reregistration eligibility process for the organophosphate pesticides.

Chapter 5

Characterizing the noncancer toxicity of mixtures using concepts from the TTC and quantitative models of uncertainty in mixture toxicity

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Abstract

This article explores the use of an approach for setting default values for the noncancer toxicity, developed as part of the Threshold of Toxicological Concern (TTC), for the evaluation of the chronic noncarcinogenic effects of certain chemical mixtures. Individuals are exposed to many mixtures where there are little or no toxicological data on some or all of the mixture components. The approach developed in the TTC can provide a basis for conservative estimates of the toxicity of the mixture components when compound-specific data are not available. The application of this approach to multiple chemicals in a mixture, however, has implications for the statistical assumptions made in developing component-based estimates of mixtures. Specifically, conservative assumptions that are appropriate for one compound may become overly conservative when applied to all components of a mixture. This overestimation can be investigated by modelling the uncertainty in toxicity standards. In this article the approach is applied to both hypothetical and actual examples of chemical mixtures and the potential for overestimation is investigated. The results indicate that the use of the approach leads to conservative estimates of mixture toxicity and therefore its use is most appropriate for screening assessments of mixtures.

Keywords: Cramer classes; DNELs; mixtures; RfD; toxicity; uncertainty

Introduction

Individuals are exposed to multiple chemicals each day. These exposures occur as exposures to discrete mixtures and concurrent exposure to multiple chemicals from multiple sources.[1] A number of researchers and organizations have developed guidance for assessing risks from exposure to mixtures;[1–5] however, performing assessments of actual mixtures is a difficult undertaking. The number of potential mixtures and the variation in the day-today composition of mixtures prevents addressing the toxicity of mixtures using traditional *in vivo* testing (whole mixture approaches).

The alternative approaches for assessing risks from mixtures are the component-based approaches.[2,5] Component-based approaches, however, require toxicity information for each component of a mixture. Available toxicity information on the components of mixtures is highly variable. Some compounds have extensive toxicity databases while other compounds may never have been tested. Such data gaps limit the use of component-based approaches. A second problem with component-based approaches is that conservative assumptions used in deriving safe levels of individual compounds are compounded when they are applied to all

of the components of a mixture. Such compounding of assumptions can lead to overestimates of mixture toxicity.[6]
Use of structure-based estimates of toxicity has the potential to support component-based approaches by providing an alternative source of toxicity data on mixture components with no toxicity data. This article investigates the feasibility of the use of an approach developed to characterize the chronic oral noncancer toxicity of many organic chemicals based on the chemicals' structures. This approach (hereafter called the "Cramer classes" approach) is a combination of work by Cramer *et al.*[7] and Munro *et al.*[8] and is part of a larger tool, the TTC.[9,10]

Probabilistic models have been used to characterize the uncertainty in estimates of safe levels of individual substances and mixtures.[6,11–14] These models allow the consideration of the impact of uncertainty in the estimates of the point of departure (POD), the use of assessment factors, and the combination of data from multiple compounds on the estimates of the toxicity of a mixture. This article uses this approach to develop "best" estimates and confidence limits for safe levels of mixtures. These estimates provide decision makers with a better understanding of the uncertainty in the estimates of mixture toxicity than simple bright-line criteria.[13] The lower confidence limits may also provide an alternative approach for setting toxicity estimates for mixtures in regulatory decision making.

This article follows the recent guidance on setting safe levels of substances proposed by the European Chemicals Agency (ECHA) in its guidance for performing risk assessments under the 2007 legislation, Registration, Evaluation, Authorization and Restriction of Chemicals.[14] This guidance describes how safe doses called derived no effect levels (DNELs) are developed. The DNEL is defined as a dose that is protective of sensitive humans and is similar to the reference dose [15] tolerable daily intake, or allowable daily intake.

The methodology presented in this article addresses chronic oral noncancer toxicity and therefore excludes the portion of the TTC approach that addresses potential carcinogens. Where one or more components of a mixture are suspected of posing carcinogenic risks, a separate assessment of such risks should be performed. Assessments of risks associated with other routes of administration are not addressed. This approach is also limited to chemicals that are similar to the chemicals represented in the Munro *et al.* database of no observed effect levels (NOELs).[8] It should not be applied to metals, organophosphorous compounds, or highly bioaccumulative substances.[9] Finally, the approach does not directly address the issue of synergy between chemicals. However, the proposed method restricts exposures to mixture components to very low doses where synergistic effects are not anticipated to occur.[16].

Modelling the Chronic Noncancer Toxicity of Mixtures Using Component-Based Approaches

Deterministic Models of Noncancer Risk

Currently, the noncancer toxicities of mixtures are evaluated using either a dose-additive or a dose-independence model.[2] The additive model assumes that each component of a mixture acts jointly by a common mechanism in a manner that is proportional to the compound's standard.[5] The independence model defines the risk from mixture as the sum of the independent effects of the components, where each component is viewed as being diluted by the entire mixture. The additive model always predicts higher toxicities for mixtures (lower DNEL values) than the independence model since it reflects the toxicity of more than one chemical.

Considerable effort has been spent to develop guidance on when, and when not, to add doses of mixture components by taking into consideration common effects, common target organs, and common metabolites.[1,5] The additive and independence models provide bounds to the uncertainty in the toxicity of the mixture that occurs as a result of uncertainty in the degree of additivity in a mixture's components. Therefore, both models are explored in this article.

The estimate of the safe dose of a mixture under the additive model of mixture toxicity, $mDNEL_A$, is given by Equation (1):

$$mDNEL_A = \frac{1}{\sum_i \frac{F_i}{DNEL_i}}, \qquad (1)$$

where F_i is the fraction of the mass of the mixture represented by the *i*th component of the mixture and $DNEL_i$ is the DNEL for the *i*th component of the mixture.[5]

Under the independence model, the safe dose of the mixture is evaluated in terms of the toxicity of each component of the mixture. Thus, setting a safe level for the mixture involves a separate assessment for each mixture component. A dose of a mixture that would only allow a safe dose of the *i*th component of the mixture ($DNEL_i$) is defined as $mDNEL_i$ (Equation (2)).

$$mDNEL_i = \frac{DNEL_i}{F_i} \qquad (2)$$

The safe dose of a mixture determined by an independence model, $mDNEL_I$, would be the lowest value of $mDNEL_i$ for the mixture's components (Equation (3)).

$$mDNEL_I = \min\left(\frac{DNEL_i}{F_i}\right) \qquad (3)$$

The contribution of individual components to an additive model of risk can be evaluated based on the toxicity weight of the component. The toxicity weight of the *i*th compound TW_i is given by the inverse of $mDNEL_i$.

$$TW_i = \frac{F_i}{DNEL_i} \qquad (4)$$

The component of a mixture with the largest TW_i value will have the largest impact on the mixture's $mDNEL_A$ and will determine the mixture's $mDNEL_I$. In this article this component is referred to as the "dominant" component of a mixture. Based on Equations (1) and (3) it is clear that when the value of TW_i for the dominant component is much larger than the sum of the TW_i, values for the remaining components, the value of $mDNEL_A$ of a mixture will approach the value of $mDNEL_I$.

Using the Cramer Classes Approach to Fill Toxicity Data Gaps

The derivation of $mDNEL_A$ or $mDNEL_I$ for a mixture requires an estimate of the safe dose of each component, $DNEL_i$. The Cramer classes approach provides a means of deriving conservative estimates of the $DNEL_i$ for chronic oral noncancer effects of organic chemicals.

Any organic compound with a known structure can be assigned into one of the three Cramer classes based on the compound's structure.[7] Munro *et al.*[8] established a conservative estimate of a POD for the chemicals in each class; see Table I. This was done by first collecting data on the no observed effect levels (NOELs) of more than 600 compounds and sorting the chemicals based on their Cramer class.

Table I. Point of Departure (POD) for each Cramer Class Based on Munro *et al.* (1996)

Cramer Class	POD (mg/kg d)
I	3
II	0.91
III	0.15

Then POD values were set based on the 5th percentiles of the log normal distributions fit to the geometric mean and geometric standard deviation of the NOELs of the chemicals in each of the three classes. In many instances, chemicals may have NOELs that exceed the POD values by orders of magnitude.

Probabilistic Models of Noncancer Standards

Background and Introduction to Probabilistic Assessments of Uncertainty in Noncancer Standards

In the last 12 years, a number of researchers have investigated the uncertainty in noncancer toxicity standards using probabilistic models.[11,12,17–25] These efforts have sought both to organize information on the uncertainties in each step of the process of establishing a standard and to quantify the resultant uncertainty in the estimate of the standard. The result of these efforts are probability density functions that describe the probability that a dose of a chemical is protective of "sensitive individuals" in an exposed population, given the limitations in the current understanding of toxicology.[11,19,20,24]

In this project, the probabilistic models that are used to characterize the uncertainty in the DNELs of individual compounds are extended to predict the uncertainty in the $mDNEL_A$. This analysis is performed in order to avoid overestimating the toxicity of a mixture. This overestimation can occur because DNELs like reference doses (RfDs) are best viewed as lower-confidence limits of estimates of dose that are protective for that chemical.[11,26] In the case of mixtures, using the DNELs for each mixture component results in an overestimation of toxicity, since the probability that all components of a mixture are as toxic as their DNELs becomes very small as the number of mixture components increases. This unintentional bias of the estimate of mixture toxicity can be avoided by modelling the uncertainty in the toxicity of the mixture and setting a consistent percentile of the distribution as the basis for the mixture's toxicity.[6]

Development of probabilistic models of mixture toxicity

As discussed by Vermeire *et al.*,[21] the inputs in the equation used to set the DNEL are modelled as uncertain variables. Equation (5) is the equation that sets the value for $DNEL_i$:

$$DNEL_i = \frac{POD_i}{\prod AF_{ki}}, \qquad (5)$$

where the POD_i is the point of departure of the critical effect for the *i*th chemical and AF_{ki} is the *k*th assessment[3] factor used in setting $DNEL_i$. Substituting this definition into Equations (1) and (3) gives:

$$mDNEL_A = \frac{1}{\sum_i \left(\frac{F_i \prod AF_{ki}}{POD_i} \right)} \qquad (6)$$

$$mDNEL_I = \min \left(\frac{POD_i}{F_i \prod AF_{ki}} \right). \qquad (7)$$

The uncertainty in $mDNEL_A$ and $mDNEL_I$ are determined by Monte Carlo modelling using distributions for the uncertainty in the PODs and assessment factors[9]. The following sections present the sources of data and approaches used for characterizing the uncertainty in the POD_i and the various types of assessment factors.

Uncertainty in POD_i

The POD_i in the $DNEL_i$ is traditionally the no observed adverse effect level (NOAEL) from a chronic animal study but could be a lowest observed adverse effect level (LOAEL), or a benchmark dose.[14] As discussed earlier, when compound specific data are not available, the POD can be conservatively estimated from the chemical's Cramer class. The uncertainty in the POD_i varies from chemical to chemical and depends on the mode of action of a chemical, the nature of the study, and the endpoint that defines the POD_i.

When the value of POD_i is based on a benchmark dose,[14] the uncertainty in the POD_i can be estimated from the confidence limits of the benchmark dose.[19]

When the POD_i is derived using the Cramer classes, the uncertainty in POD_i is determined based on the distribution of the NOELs associated with each of the Cramer classes. This approach is a logical extension of the assumption that the NOELs identified by Munro *et al.*[8] are a representative sample of the chemical in each Cramer class and the 5th percentile of the NOELs will be a conservative estimate of the NOELs of the substances in each class. Under this assumption,

[9] Other authors have used the terms "extrapolation," "safety," "adjustment," or "uncertainty" factors for these parameters.

variation in the NOELs becomes a measure of the uncertainty in the NOEL of a chemical that is a random sample of the population of chemicals in each of the classes. In this article, the distribution for each Cramer class is developed by sampling (with replacement) the values of the NOELs for the chemicals in each Cramer class as presented in the appendix of Munro *et al.*[8]

If the POD_i of a chemical is based on a NOAEL or LOAEL, the uncertainty in the POD_i is more difficult to estimate. Brand *et al.*[27,28] demonstrated that the uncertainty in NOAEL of a chemical in a specific study is a function of the shape of the underlying dose-response curve, dose spacing, background level of response, and the number of animals tested.

Distributions for assessment factors (AF_{ki})

There are a number of assessment factors that are used in setting DNELs. These include:

1. Interspecies uncertainty.
2. Interindividual uncertainty.
3. Exposure duration extrapolation.
4. Database adequacy.
5. LOAEL to NOAEL adjustment.

The number and specific nature of the assessment factors used in assessing each component of a mixture are determined by the toxicity data available for the component.

The concept of replacing the single value of an uncertainty factor with a distribution has been described by Brand *et al.*[27,28] and Swartout *et al.*[11] The distributions for each uncertainty factor are based on the interchemical variation in the size of the adjustment in dose required to go from one measure of toxicity to another. The interspecies factor is based on going from a POD in animals to a POD in typical humans. The interindividual factor is based on going from a POD for a population of typical humans to a POD in a population of sensitive humans. Similar distributions are set for the subchronic and database assessment factors. Estimates of the distributions can be based on policy considerations [11,20] or empirical data.[19,30]

Table II. Allometric Scaling Factor for Different Species of Test Animals (ECHA, 2008)

Mouse	7
Rat	4
Dog	1.4
Monkey	2

Interspecies uncertainty factor

The interspecies uncertainty factor has been the subject of a number of publications.[29–33] In this article, the distribution for this factor is based on the two-part approach (allometric scaling plus a residual uncertainty term) proposed by Renwick[34] and Schneider *et al.*[33] The allometric term is a function of the test animal used in the study that set the NOAEL. Table II presents the allometric scaling factors proposed in recent REACH guidance[14] and in Vermeire *et al.*[27]

The distribution for the residual uncertainty term is taken from Schneider *et al.*,[33] who proposed a log normal distribution with a geometric mean and geometric standard deviation of 0.97 and 3.23, respectively.

Interindividual uncertainty factor

Data on interindividual variation and the size and variation of the factor required to address it has been quantitatively evaluated by Schneider *et al.*[22] They proposed the use of a displaced log normal distribution with a displacement of 1, a geometric mean and geometric standard deviation of 2.31 and 3.57, respectively.

While Schneider *et al.* state that this distribution will protect 90% of exposed individuals, the actual fraction protected is likely to be higher. The reason for this is that Schneider *et al.* assumed that the intraindividual is the ratio of dose affecting a typical person (an EC_{50}) to a dose that affects some lower fraction of the population (EC_{10}, EC_5, or EC_1,). As discussed in Price *et al.*,[35] the ratio should be based on the ratio of the dose that is protective of a population of typical individuals and the dose that is protective of a population of sensitive individuals. Thus the ratio should be assumed to be applied to some larger fraction of the total population (perhaps EC_{80} or EC_{90}) rather than the EC_{50}. Starting from this higher fraction, the degree of protection resulting from the distribution of interindividual uncertainty factors will be considerably greater than 90%.

Exposure duration extrapolation

Data on the uncertainty in extrapolating from subchronic to chronic PODs has been investigated by both Bokkers and Slob[36] and Kalberlah *et al.*[32] Kalberlah *et al.* used ratios of NOAELs to investigate the effect of extrapolating from subchronic to chronic doses. Bokkers and Slob used ratios of benchmark doses to investigate the assessment factor. This approach avoids a portion of the "noise" in the measurements created by the use of NOAEL and thus provides a better estimate of the true distribution. Bokkers and Slob reported that the distribution was log normal with a geometric mean and geometric standard deviation of 1.7 and 2.9, respectively. Data on the uncertainty in extrapolating from subacute to chronic POD have been investigated most recently by Schneider *et al.* [37] They reported that the distribution was log normal with a geometric mean and geometric standard deviation of 4.14 and 2.03, respectively.

LOAEL to NOAEL assessment factors

There is limited data on the interchemical variation in the extrapolation of the LOAEL to NOAEL and the database adequacy factors. In this article the policy-based distribution proposed by Swartout *et al.* [11] is used. If the standards for a substance use a full factor of 10 then the Swartout distribution is used; if the standards use a partial factor (3 rather than 10), then the square root of values from the Swartout distribution are used.

Example Applications of the Approach

The use of probabilistic models and the Cramer classes is applied to two hypothetical mixtures that were selected to demonstrate the approach and to demonstrate how the information generated by the approach could be used to evaluate the toxicity of mixtures. The approach is then applied to a series of actual mixtures of chemicals reported measured in samples of surface waters.[38]

Two hypothetical mixtures

The first of the two mixtures is composed of equal amounts of 10 chemicals. There are no toxicity data available for any of the chemicals. All of the chemicals' structures fall into Cramer class III.

The assessment factors used for the chemicals are the interspecies and the interindividual assessment factors. The species most frequently used in the studies reviewed by Munro *et al.*[8] is the rat. Therefore, the allometric component of the interspecies adjustment factor used for these compounds was 4. These assumptions are consistent with the 100- fold safety factor applied to the PODs in the TTC.[10]

This mixture was chosen to illustrate how the toxicity of a mixture can be characterized where there are no toxicity data available on the mixture's components. The mixture is also intended to maximize the differences between the independence and additive models since in this mixture 10 chemicals make identical contributions to the mixture's toxicity (have the same TW_i values).

The second mixture is composed of toluene, a compound with known toxicity and two compounds with no toxicity data. The first compound's structure falls into Cramer class III and the second into Cramer class II. The fractions of the components by weight are 50% toluene, 30% the Cramer class III substance, and 20% the Cramer class II substance. There is a benchmark dose for toluene and the uncertainty in that value is used for the uncertainty in the POD.

The purpose of Example 2 is to demonstrate how mixtures with a combination of known and unknown toxicities can be accommodated in one assessment. Unlike Example 1, the components vary in toxicity and weight fraction and thus have different TW_i values.

Mixtures of chemicals measured in surface water

Example 3 is a real-world application of the approach to a set of 48 mixtures. These mixtures are taken from monitoring data of contaminants found in samples of surface waters in the State of Minnesota.[38] This study analyzed for a wide range of chemicals known to occur in wastewater discharges. In this study a total of 48 samples of surface water were identified with measurable levels of two or more of these compounds. The average number of compounds in a mixture is 5.6 and ranged from 2 to 23. A total of 45 unique compounds were identified in one or more of these mixtures (see Table III).

This data set was selected as an example of the type of mixtures that occur in the environment and include a wide range of chemicals with differing levels of toxicity. In this study all of the nondetects measurements were set to zero. This assumption was made to simplify the example and may not be appropriate in an actual assessment of the risks posed by the contaminants in these waters.

Toxicity data

A search for chronic noncancer standards for the chemicals was performed using the sources listed in Table IV. The first database searched was the Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) assessment database, and if available, the standards from IRIS are used for this exercise. If no value was available from IRIS then a standard is taken from the other sources. Chronic noncancer standards are identified for toluene and 31 of the 44 chemicals in the Lee *et al.* data.

If no adequate data were identified for a chemical, the Cramer class for the chemical was used to define the POD values for the chemical. Cramer classes for the chemicals were determined using the OECD[10] (Q)SAR Application Toolbox (proof of concept version 0.6 computer software package, LMC, Bulgaria).

During this search it became obvious that the methods for setting the standards varied with the source of the standards and the date that the standards were established. The values for some assessment factors used in setting some of the standards are not consistent with more recent approaches for setting standards. Therefore, in this project it was decided to use the PODs and the specific assessment factors used to establish the compounds' original standards, but to calculate new standards ($DNEL_i$) using guidance developed by the ECHA for setting DNELs.[14]

Some of the sources of the standards specify a database assessment factor to account for missing data. The definition of which data are lacking, however, depends on the set of data required by the regulatory body setting the standard. These requirements differ across different standard-setting bodies. Therefore, this factor was omitted in the deterministic and probabilistic analyses of the compounds. Application of this factor should be considered when applying this methodology in an actual assessment.

Table III presents the Cramer classes and toxicity data identified for the compounds. The resulting deterministic values of $DNEL_i$ for each compound are also listed. The $DNEL_i$ values ranged from 0.0005 to 20 mg/(kg d).
Data necessary to characterize the uncertainty in the LOAELs and NOAELs for the 45 substances were not readily available for the substances.

[10] http://oasis-lmc.org/?section=software

Table III. Compounds and Toxicity data Used in Example Mixtures

Compound	Cramer Class	Source of POD and Assessment Factors	POD (mg/(kg d))	Species	Duration	AF Inter-species	AF Intra-species	AF Dura-tion	AF LOAEL to NOAEL	$DNEL_4$ (mg/(kg d))
1-methylnaphthalene	III	ATSDR	71.6	Mouse	Chronic	17.5	10	1	3	0.14
2-methylnaphthalene	III	IRIS	3.5	Mouse	Chronic	17.5	10	1	1	0.020
3-β-coprostanol	III	Cramer class	0.15			10	10	1	1	0.0015
3-methyl-1H-indole (skatol)	III	Cramer class	0.15			10	10	1	1	0.0015
4-octylphenol	II	Cramer class	0.91			10	10	1	1	0.0091
5-methyl-1H-benzotriazole	III	Cramer class	0.15			10	10	1	1	0.0015
Acetyl-hexamethyl-tetrahydro-naphthalene	II	HERA	5	Rat	Subchronic	10	10	2	1	0.0250
Anthraquinone	III	Cramer class	0.15			10	10	2	1	0.0015
Benzophenone	III	NTP	15	Rat	Chronic	10	10	1	3	0.050
β-sitosterol	III	EU SCF	4000	Rat	Subchronic	10	10	2	1	20
β-stigmastanol	III	Cramer class	0.15			10	10	1	1	0.0015
Bisphenol-A	III	IRIS	50	Rat	Subchronic	10	10	2	1	0.25
Bromacil	III	EPA RED	5	Dog	Chronic	3.75	10	1	1	0.13
Bromoform	III	IRIS	17.9	Rat	Subchronic	10	10	2	1	0.09
Caffeine	III	OECD	150	Rat	Subchronic	10	10	2	1	0.75
Carbazole	III	Cramer class	0.15			10	10	1	1	0.0015
Cholesterol	I	Cramer class	3			10	10	1	1	0.030
Cotinine	III	Cramer class	0.15			10	10	1	1	0.0015
Diazinon	III	EPA RED	0.02	Dog	Chronic	3.75	10	1	1	0.0005
D-limonene	I	WHO	10	Rat	Subchronic	10	10	2	1	0.05
Fluoranthene	II	IRIS	125	Mouse	Subchronic	17.5	10	2	1	0.36
Hexahydrohexamethyl-cyclopentabenzopyran	III	Cramer class	0.15			10	10	1	1	0.0015
Indole	III	Cramer class	0.15			10	10	1	1	0.0015
Isophorone	II	IRIS	150	Dog	Subchronic	3.75	10	2	1	2.0
Menthol	I	OECD	188	Rat	Chronic	10	10	1	1	1.9
Methyl salicylate	III	Cramer class	0.15			10	10	1	1	0.0015
Metolachlor	III	IRIS	15	Rat	Chronic	10	10	1	1	0.15
Naphthalene	III	IRIS	71	Rat	Subchronic	10	10	2	1	0.71
N,N-diethyl-meta-toluamide	III	EPA RED	100	Rat	Chronic	10	10	1	1	1.0
Nonylphenol diethoxylate	II	ESIS	15	Rat	Multigeneration	10	10	2	3	0.025
para-nonylphenol	II	Cramer class	0.91			10	10	1	1	0.0091
Pentachlorophenol	III	IRIS	3	Rat	Chronic	10	10	1	1	0.03
Phenanthrene	III	Cramer class	0.15			10	10	1	1	0.0015
Phenol	I	IRIS	93	Rat	Subchronic	10	10	2	1	0.47
Prometon	III	IRIS	15	Rat	Subchronic	10	10	2	1	0.075
Pyrene	III	IRIS	75	Mouse	Subchronic	17.5	10	2	1	0.21
Tetrachloroethylene	III	IRIS	14	Mouse	Subacute	17.5	10	6	1	0.04
Toluene	I	IRIS	238	Rat	Subchronic	10	10	2	1	1
Tri(2-butoxyethyl) phosphate	III	MHLW	100	Rat	Subacute	10	10	6	1	0.17
Tri(2-chloroethyl) phosphate	III	NTP	44	Rat	Chronic	10	10	1	3	0.15
Tributyl phosphate	III	OECD	9	Rat	Chronic	10	10	1	1	0.090
Triclosan	III	EPA RED	30	Monkey	Chronic	5	10	1	1	0.60
Tri(dichlorisopropyl) phosphate	III	EPA HPV	5	Rat	Chronic	10	10	1	1	0.050
Triethyl citrate (ethyl citrate)	III	WHO	2000	Rat	Chronic	10	10	1	1	20
Triphenyl phosphate	III	OECD	161	Rat	Subchronic	10	10	2	1	0.81

Table IV. Sources of Toxicity Data Considered in This Study

US EPA Integrated Risk Information System	http://cfpub.epa.gov/ncea/iris/index.cfm
ATSDR	http://www.atsdr.cdc.gov/toxpro2.html
BGChemie chemicals assessments	http://www.bgchemie.de/webcom/show_page.php/_c-86/_nr-1/i.html
BUA substance reports	http://www.gdch.de/fowi/archiv/bua/berichte/bua_stoffecas.htm
European Substances Information System (ESIS)	http://ecb.jrc.it/esis/
EU SCF	http://ec.europa.eu/food/fs/sc/scf/reports_en.html
HERA	http://www.heraproject.com/RiskAssessment.cfm
MHLW Japanese Toxic Chemicals Database	http://wwwdb.mhlw.go.jp/ginc/
JECFA monographs	http://www.inchem.org/pages/jecfa.html
OECD Integrated HPV database	http://cs3-hq.oecd.org/scripts/hpv/index.asp
UNEP OECD SIDS database	http://www.chem.unep.ch/irptc/sids/OECDSIDS/sidspub.html
US EPA HPV challenge	http://cfpub.epa.gov/hpv-s/
US EPA RED	http://www.epa.gov/pesticides/reregistration/status.htm
US NTP database	http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm
WHO UNEP International Program on Chemical Safety	http://www.who.int/IGHRC/publications/cicad/en/index.html

In this project an estimate of the uncertainty in NOAELs and LOAELs was made by using a triangular distribution where the reported NOAEL or LOAEL dose is the most likely value of the POD_i and the max and min are one-third and three times the value of the NOAEL or LOAEL. The selection of this distribution range is based on the observation that the dose spacings in most animal studies have values of three or less.

Analyses performed

The following analyses were performed for all of the example mixtures.

1. Determination of deterministic values of $mDNEL_A$ and $mDNEL_I$.
2. Calculation of the ration of $mDNEL_A$ and $mDNEL_I$.
3. Determination of the uncertainty in the estimates of the mixtures' toxicities under the additive and independence models ($mDNEL_A$ and $mDNEL_I$) using Monte Carlo analysis

The results of the uncertainty assessments are presented using the median values (values equally likely to be over- or underestimates of the true threshold in sensitive humans) and the 90% upper and lower confidence limits of the distribution (the 5th and 95th percentiles). The Monte Carlo analysis used Latin hypercube sampling and a total of 10,000 iterations were performed for each mixture. This number of iterations was empirically determined to produce stable estimates of the reported values. Each of the 48 mixtures was evaluated to determine which component was dominant in the mixture using Equation (4).

Fifteen of the 48 mixtures only contained compounds that had existing standards and thus did not require the use of the Cramer classes approach to estimate their toxicities. These mixtures provide an opportunity to demonstrate the degree of conservative bias associated with the use of the Cramer classes. The values of $mDNEL_A$ and $mDNEL_I$ for the 15 mixtures were determined using both the actual data for the substances and using the POD for Cramer classes for each of the mixture's components. The results using the two approaches were then compared to determine if the use of the PODs from Cramer classes were protective.

Results

Examples 1 and 2

The results for the first two examples are given in Table V and Figs. 1 and 2

Table V. Estimates of Mixture toxicity for Examples 1 and 2 (mg/kg/d))

	Deterministic Model Results		Probabilistic Model Results					
	Additive Model	*Independence Model*	*Additive Model*			*Independence Model*		
Calculated Using:	Equation (1)	Equation (3)	Equation (5)			Equation (6)		
			5th Percentile	Median	95th Percentile	5th Percentile	Median	95th Percentile
Example 1	0.0015	0.015	0.0034	0.081	0.87	0.0039	0.12	2.0
Example 2	0.0045	0.0050	0.014	0.73	13	0.015	0.94	20

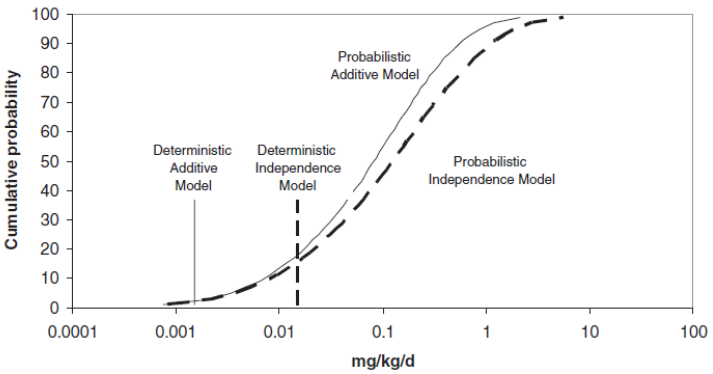


Figure 1. Toxicity of mixture 1 under independence and additive models using deterministic and probabilistic approaches

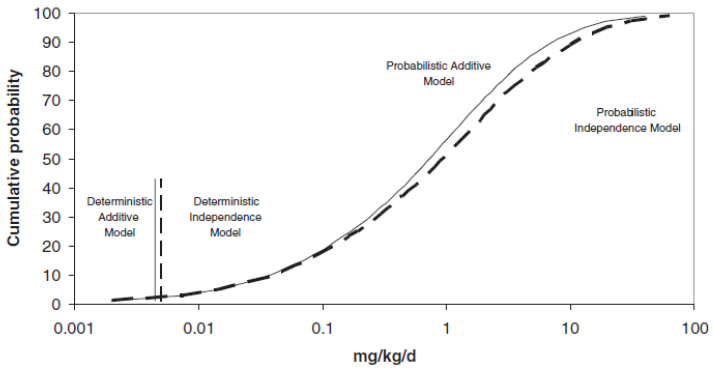


Figure 2. Toxicity of mixture 2 under independence and additive models using deterministic and probabilistic approaches

Example 1 is a mixture of equal amounts of 10 compounds that fall into Cramer class III. The deterministic values of $mDNEL_A$ and $mDNEL_I$ for this mixture differ by a factor of 10. The lower percentiles of the uncertainty distributions for independence and additive models, however, differ by less than 10%. At the higher percentiles of the uncertainty distributions the additive and independence models diverge. At the 95th percentile they differ by a factor of 2. The 5th percentiles of uncertainty distributions for the additive and independence models are approximately 2.4-fold higher than the corresponding deterministic value of $mDNEL_A$ and 4.1-fold lower than the deterministic value of $mDNEL_I$.

The median estimate of the uncertainty distribution of the additive model is 54-fold higher than the deterministic estimate and the median estimate of the uncertainty independence model distribution is 8-fold higher than the deterministic estimate.

Table VI. Basis for the POD for Dominant Component of Each Mixture Ranked by the Additive Toxicity of Each Mixture (mg/(kg/d))

$mDNEL_A$	Dominant Component	Basis for POD for the Dominant Component
8.16E-03	5-methyl-1H-benzotriazole	Cramer class
3.57E-02	3-β-coprostanol	Cramer class
4.01E-02	β-stigmastanol	Cramer class
2.68E-01	3-β-coprostanol	Cramer class
3.87E-01	3-β-coprostanol	Cramer class
1.60E+00	3-methyl-1H-indole	Cramer class
1.10E-02	3-β-coprostanol	Cramer class
4.88E-03	3-β-coprostanol	Cramer class
3.32E-01	β-stigmastanol	Cramer class
2.06E-01	4-normal-octylphenol	Cramer class
1.93E-02	3-β-coprostanol	Cramer class
6.42E-02	Diazinon	Compound specific
3.13E-02	3-methyl-1H-indole	Cramer class
1.13E-01	Diazinon	Compound specific
3.64E-02	Nonylphenol diethoxylate	Compound specific
1.22E+00	*para*-nonylphenol	Cramer class
6.44E-01	3-β-coprostanol	Cramer class
2.72E-02	Nonylphenol diethoxylate	Compound specific
9.69E-01	Cholesterol	Cramer class
1.27E-02	AHTN	Compound specific
6.64E-02	*para*-nonylphenol	Cramer class
5.04E-02	Cholesterol	Cramer class
7.91E-02	Cholesterol	Cramer class
6.30E-02	3-Methyl-1H-indole	Cramer class
3.43E-01	3-Methyl-1H-indole	Cramer class
5.07E-02	Cholesterol	Cramer class
6.86E-02	3-Methyl-1H-indole	Cramer class
5.13E-03	Cholesterol	Cramer class
1.09E-02	*para*-nonylphenol	Cramer class
1.18E-02	D-limonene (1)	Compound specific
3.30E-02	D-limonene (1)	Compound specific
8.81E+00	Cholesterol	Cramer class
4.79E-02	Cholesterol	Cramer class
4.68E-02	Cholesterol	Cramer class
1.48E-01	3-Methyl-1H-indole	Cramer class
7.84E+00	Cholesterol	Cramer class
4.48E-02	Tributyl phosphate	Compound specific
1.37E+00	Phenol	Compound specific
3.11E-03	Phenol	Compound specific
1.92E-02	Metolachlor	Compound specific
6.64E-03	Phenol	Compound specific
9.14E-03	Phenol	Compound specific
4.16E-03	Metolachlor	Compound specific
2.42E-02	Isophorone	Compound specific
4.63E-03	Isophorone	Compound specific
5.10E-03	Isophorone	Compound specific
4.56E-03	Metolachlor	Compound specific
9.97E-03	Metolachlor	Compound specific

Example 2 is a mixture of three chemicals with differing toxicity weights. The toxicity of the mixture is dominated by the toxicity of one component (30% of the mixture and falls into Cramer class III). Because of this dominance there is little

difference between the estimates of mixture toxicity under the additive and independence models as measured by either the deterministic or the probabilistic approaches. The lower 5th percentiles of the uncertainty distributions of $mDNEL_A$ and $mDNEL_I$ are threefold higher than the deterministic estimates and the medians are roughly 170-fold higher.

Actual mixtures of low-level contaminants in surface water

Figures. 3 and 4 present the results of the deterministic and probabilistic modelling of $mDNEL_A$ and $mDNEL_I$ for each of the 48 mixtures. Each figure gives the deterministic value, the median and 90% confidence limits for the mixtures. In each of these figures, the mixtures are ranked based on the deterministic values of toxicity. As shown by the $mDNEL_A$ values, the toxicities of the mixtures are highly variable, ranging from less than 0.01 to approximately 10 mg/(kg d).

The probabilistic models of the uncertainty in $mDNEL_A$ and $mDNEL_I$ predicted median values are considerably higher than the deterministic predications. The average of the 48 median values is 55-fold higher for the additive model and 70-fold higher for the independence model. There was a much closer fit between the deterministic estimate and the lower 90% confidence limits. The average of the confidence limits of both the independence and additive models across the 48 mixtures are 1.3-fold higher than the deterministic values . . .

While 31 of the 44 compounds did not require the use of the Cramer classes to set values of $DNEL_i$, the dominate compounds in the majority of the mixtures (30 of the 48) were compounds where Cramer classes were used (Table III). In addition, the mixtures with the lowest values of $mDNEL_A$ (most toxic) were dominated by compounds where the Cramer classes were used to set the values of $DNEL_i$. This finding is not surprising since the estimates of toxicity produced by the Cramer class approach are intended to be highly conservative and if a mixture component set using the Cramer class is present as a significant fraction of the mixture, that component would be expected to drive the mixture's toxicity.

Additional evidence of the conservative nature of the Cramer class approach is given in Table VII. This table presents the toxicity values that would have occurred if the Cramer class had been used instead of the actual toxicity data for the 15 mixtures where data are available on all mixture components.

Figure 3 Toxicity of 48 mixtures of contaminants under additive models: deterministic values and median and 90% confidence limits of the uncertainty distribution.

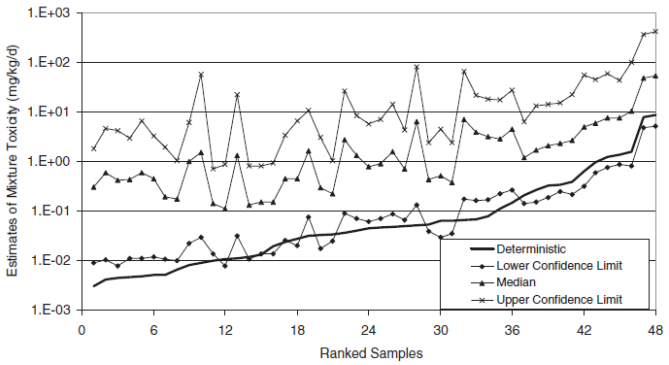
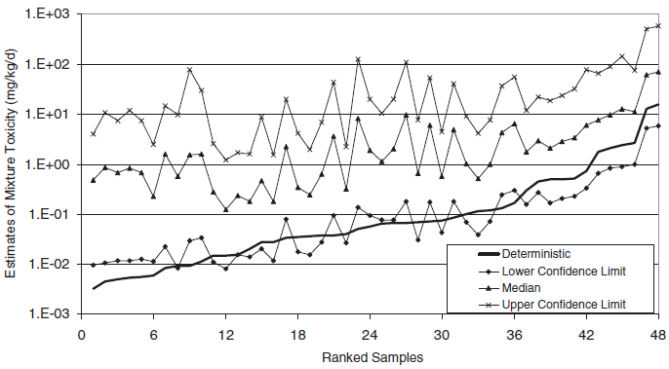


Figure 4. Toxicity of 48 mixtures of contaminants under independence model: deterministic values and median and 90% confidence limits of the uncertainty distributions.



$mDNEL_A$ Based on Compound-Specific Toxicity Data	$mDNEL_A$ Based on Cramer Class	Ratio of the Two Approaches	$mDNEL_I$ Based on Compound-Specific Toxicity Data	$mDNEL_I$ Based on Cramer Class	Ratio of the Two Approaches
2.70E-02	6.30E-03	4	2.70E-02	1.00E-02	3
4.80E-02	3.10E-03	16	5.60E-02	3.40E-03	17
6.30E-02	5.10E-03	12	7.00E-02	7.10E-03	10
6.40E-02	4.50E-03	14	1.20E-01	1.20E-02	10
2.10E-01	2.40E-03	88	3.00E-01	5.00E-03	60
2.70E-01	1.50E-02	18	4.90E-01	3.00E-02	17
3.30E-01	1.40E-02	23	4.90E-01	3.20E-02	16
3.40E-01	1.50E-03	230	4.40E-01	2.30E-03	200
3.90E-01	1.10E-02	36	5.10E-01	1.60E-02	33
6.40E-01	1.60E-02	39	7.30E-01	2.50E-02	29
9.70E-01	6.30E-03	150	1.70E+00	1.00E-02	180
1.20E+00	7.20E-03	170	2.10E+00	9.60E-03	220
1.40E+00	4.00E-03	340	2.70E+00	6.50E-03	410
1.60E+00	4.90E-03	330	2.40E+00	8.90E-03	270
8.80E+00	1.50E-03	5,900	1.60E+01	1.50E-03	10,000

Table VII. Impact of the Use of Cramer Class to Estimate Mixture Toxicity (mg/(kg d))

Figure 5. Determines estimates of toxicity of 48 mixtures of contaminants found in surface water samples

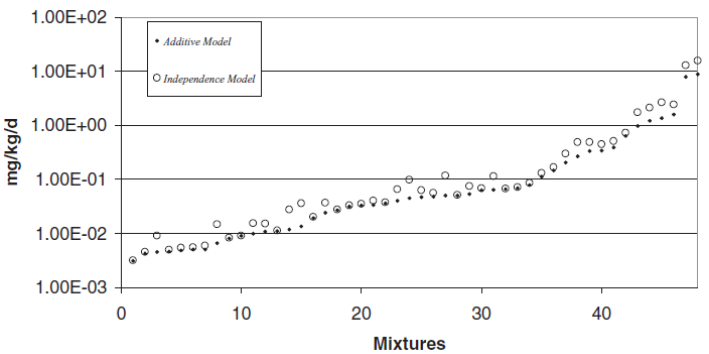
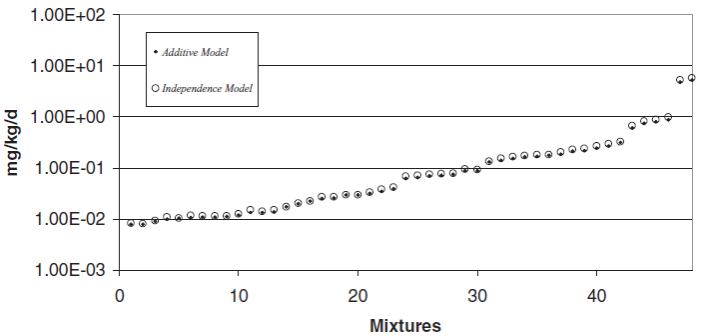


Figure 6. Lower 90% confidence limits (5th percentiles of uncertainty distributions) of the toxicity of 48 mixtures of contaminants found in surface water samples



As Table VII shows, had toxicity data not been available, the estimates of the mixture's toxicities would have been 4-fold to 5,000-fold lower for the additive model and 3-fold to 10,000-fold for the independence model.

Fig. 5 presents a comparison of the estimates of the mixture's toxicities under additive and independence models. Figure 6 presents the same comparisons for the lower confidence limits (5th percentiles) of the uncertainty distributions of the additive and independence models. In both figures the compounds have been ranked from the lowest to the highest values of the additive model.

Figure 5 shows that while the deterministic values of $mDNEL_I$ are always higher than $mDNEL_A$, the differences are small. The actual values of the ratios of the doses under independence and additive models range from 1.0003 to 2.9 and average 1.4. These small differences indicate that the toxicities of most of the 48 mixtures are dominated by a single compound (such as in example mixture 2).

Even in mixtures where the additive and independence models give different answers, the probabilistic version of the additive and independence models produce very similar results. For example, the surface water sample with the largest difference between the deterministic $mDNEL_I$ and $mDNEL_A$ values differs by 2.7-fold and the lower confidence limits for the samples $mDNEL_I$ and $mDNEL_A$ only differ 1.1-fold.

Discussion

The approach and demonstration examples in this article indicate that Cramer classes can support the development of component-based estimates of mixture toxicity for mixtures where there are little or no toxicity data. Using Cramer classes it is possible to characterizing the chronic noncancer toxicity of mixtures composed of any number of components providing that

- the weight fraction of each component is known;
- the chemical structures of the components are known; and
- the chemical structures of the components are reasonably represented by the chemicals in the Munro *et al*.[8] database.

In addition, Cramer classes can be used to fill in missing data for mixtures where data exist on a portion of a mixture's components. Application of the TTC to mixtures has been discussed by Kroes *et al.*[39]

When dealing with complex mixtures of diverse chemicals, assessment using the TTC approach should focus on the exposure to a "marker" compound or major compound which represents a high proportion of the mixture and is in the highest Cramer class of the known constituents of the mixture.

The approach proposed here differs from Kroes *et al.* by: one, only addressing the noncancer portion of the TTC; two, introducing consideration of the fraction of each component in the mixture into the assessment; and, three, considering the impact of multiple components in the mixture's toxicity. Thus this approach builds on the approach suggested by Kroes *et al.* and brings consideration of additional information into the process of setting toxicity estimates for mixtures.

The values of toxicity produced by use of Cramer classes, however, have a significant potential to overestimate the toxicity of mixtures. When the toxicity of the dominant component of a mixture (has the largest TW_i) is defined using the Cramer classes, the toxicity of the mixture can be overestimated by up to three orders of magnitude. When such components are not the dominant compound, the impact of using the Cramer classes is much less of a concern. This suggests that when a Cramer class is used to provide toxicity estimates for one or more components, the user should determine if any of the components become the dominant component of the mixture. If this is the case then the prediction of the toxicity of the mixture should be taken with caution.

This limitation does not mean that such estimates are without value. Such conservative estimates may find considerable use in screening out low-risk mixtures. Such uses would be consistent with the philosophy of the TTC[10]

This study also investigated the use of probabilistic models of the uncertainty in the estimates of mixture toxicity. The analyses confirm that the deterministic predictions are conservative. The concern that the application of conservative assumptions to multiple chemicals would overestimate the toxicity of mixtures did not prove to be an important issue since most mixtures had their toxicities driven by one or two chemicals.
However, the uncertainty analysis still provides significant benefits. As discussed by the National Research Council[13] and ECHA,[14] the use of probabilistic techniques improves the risk assessment process by providing the risk manager with a context for single value measures of toxicity. For example, providing the median value of the uncertainty distribution in the mixture's DNELs discloses the degree of precaution built into the deterministic estimates. In addition, the use of the 5th percentile of the uncertainty distributions provides a more objective and consistent measure of toxicity across different mixtures. This consistency could improve comparative risk determinations.

Finally, the uncertainty analysis provides a quantitative guide to the impact of exceeding the mixture's DNEL. As shown in Figs. 3 and 4, the differences between the median value and the deterministic value vary across mixtures. One of the 48 mixtures had a median value of additive toxicity that was 306-fold higher than the deterministic value while another had a value that was only 5.4- fold higher. Clearly, a dose of the first mixture that exceeds that mixture's deterministic DNEL by a factor of 10 is much less worrisome than a dose of the second mixture exceeding that mixture's DNEL by a similar amount.

The finding that additive and independence models produce similar results in the 48 surface water samples was unexpected. Historically, independence models are thought to provide different results than additive models.[1] In addition, many components of the mixtures were assigned the same toxicity values (they were evaluated using the Cramer classes and fell in the same category). This should have increased the difference between both the independence and additive models, as demonstrated with Example 1. If the pattern of one compound dominating the toxicity of a mixture that was observed in 48 mixtures of Example 3 occurs in other environmental mixtures, then the decision of whether to assume independence or additivity may not be a critical issue in assessing the risks of environmental mixtures. However, the use of both the independence and the additive models is still recommended to determine the value of refining the determination of those components of a mixture add and those that do not add.

The finding that the lower confidence limits of independent and additive models produced similar estimates of mixture toxicity is also an interesting finding. This

phenomena was observed in both Example 1 where the independent and additive model differed by a factor of 10 and in the 48 surface water mixtures.

This observation is likely to be related to the uncertainty in the estimates of the toxicity of the individual mixture components. This uncertainty implies that even when compounds appear to be making similar contributions to a mixture's toxicity in a deterministic analysis, one of the substances is actually dominating the mixture's toxicity. This finding should be investigated further in the future.

Conclusions and Future Work

This project has demonstrated that a combination of existing models in mixture toxicity, the Cramer classes, and probabilistic modelling of the uncertainty in noncancer chronic standards provide a novel and useful method of characterizing mixtures' toxicity. Such characterizations are likely to be biased toward overestimating toxicity and thus the approach may be more relevant for screening decisions.

Acknowledgments

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References

1. U.S. EPA. Concepts, methods and data sources for cumulative health risk assessment of multiple chemicals, exposures and effects: A resource document. Cincinnati, OH: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, 2007. [EPA/600/R-06/013F].
2. Interdepartmental Group on Health Risks from Chemicals (IGHRC). Chemical mixtures: A framework for assessing risks (Version 6, April 2007) [Internet]. 2007. [cited 2008 December 1]. Available at: http://ieh. cranfield.ac.uk/IGHRC/mixtures document.pdf.
3. Hertzberg RC, Teuschler LK. Evaluating quantitative formulas for dose–response assessment of chemical mixtures. Environmental Health Perspectives, 2002; 110(Suppl 6):965– 970.
4. Agency for Toxic Substances and Disease Registry (ATSDR). Guidance Manual for the Assessment of Joint Toxic Actions of Chemical Mixtures. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, 2004.
5. U.S. EPA. Supplementary guidance for conducting health risk assessment of chemical mixtures. In: Risk Assessment Forum, Washington, DC: U.S.

Environmental Protection Agency, Office of Research and Development, 2000. [EPA 630/R-00-002].
6. Carlson-Lynch H, Price PS, Swartout JC, Dourson ML, Keenan RE. Application of Quantitative information on the uncertainty in the RfD to noncarcinogenic risk assessments. Human and Ecological Risk Assessment, 1999; 5(3):527–546.
7. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard— A decision tree approach. Food and Cosmetics Toxicology, 1978; 16(3):255–276.
8. Munro IC, Ford RA, Kennepohl E, Sprenger JG. Correlation of structural class with no-observed-effect levels: A proposal for establishing a threshold of concern. Food and Chemical Toxicology, 1996; 34(9):829–867.
9. Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A. Structure-based thresholds of toxicological concern (TTC): Guidance for application to substances present at low levels in the diet. Food and Chemical Toxicology, 2004; 42(1):65–83.
10. Barlow S. Threshold of Toxicological Concern (TTC)—A Tool for Assessing Substances of Unknown Toxicity Present at Low Levels in the Diet. Washington, DC: International Life Sciences Institute, 2005.
11. Swartout JC, Price PS, Dourson ML, Carlson-Lynch HL, Keenan RE. A probabilistic framework for the reference dose (probabilistic RfD). Risk Analysis, 1998; 18(3):271–282.
12. Vermeire T, Stevenson H, Peiters MN, Rennan M, Slob W, Hakkert BC. Assessment factors for human health risk assessment: A discussion paper. Critical Reviews in Toxicology, 1999; 29(5):439–490.
13. NRC. Science and Decisions: Advancing Risk Assessment. Committee on Improving Risk Analysis Approaches Used by the U.S. EPA, Washington, DC: National Research Council, 2008.
14. European Chemicals Agency (ECHA). Characterisation of dose [concentration]-response for human health. In: Guidance on Information Requirements and Chemical Safety Assessment. Helsinki, Finland: European Chemicals Agency, 2008.
15. U.S. EPA. Reference Dose (RfD): Description and use in health risk assessments: Background Document 1A [Internet]. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. 1993. Available at: http://www.epa.gov/IRIS/rfd.htm, Accessed on December 1, 2008.
16. Könemann WH, Pieters MN. Confusion of concepts in mixture toxicology. Food and Chemical Toxicology, 1996; 34(11– 12):1025–1031.
17. Price PS, Keenan RE, Swartout JC, Gillis CA, Dourson MC. A Monte Carlo based approach for modelling noncarcinogenic dose response rates. Paper presented at Society for Risk Analysis Annual Meeting, December 3–5, Sheraton Waikiki Hotel, Honolulu, HI, 1995.

18. Baird SJS, Cohen JT, Graham JD, Shlyakhter AI, Evans JS. Noncancer risk assessment: A probabilistic alternative to current practice. Human and Ecological Risk Assessment, 1996; 2(1):79–102.
19. Slob W, Pieters MN. A probabilistic approach for deriving acceptable human intake limits and human health risks from toxicological studies: General framework. Bilthoven (The Netherlands): RIVM, 1997. [Rapport 620110005].
20. Slob W, Pieters MN. A probabilistic approach for deriving acceptable human intake limits and human health risks from toxicological studies: General framework. Risk Analysis, 1998; 18(6):787–798.
21. Vermeire T, Stevenson H, Peiters MN, Rennan M, Slob W, Hakkert BC. Probabilistic assessment factors for human health risk assessment: A practical guide. Bilthoven, The Netherlands: RIVM, 1991. [TNO report V3489].
22. Schneider K, Schuhmacher-Wolz U, Hassauer M, Darschni KS, Elmshauser E, Mosbach-Shulz O. A probabilistic effect assessment model for hazardous substances at the workplace. Regulatory Toxicology and Pharmacology, 2006; 44(2):172-81.

Chapter 6

Modelling the chronic non-cancer effects of mixtures of migrants using Cramer classes and quantitative models of uncertainty

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Abstract

This paper presents a quantitative tool for assessing the toxicity of mixtures of substances that enter food from packaging materials (migrants). These estimates of mixture toxicity are believed to be conservative (the actual toxicity of the mixture will be less than the predicted estimates). These conservative estimates can be used to screen out mixtures of migrants having a low likelihood of causing adverse non-carcinogenic effects. The approach used in the tool is based on the concept of Cramer classes and existing models of mixture toxicity. The tool takes advantage of recent advances in modelling the uncertainty in estimates of safe doses of individual chemicals and mixtures of chemicals and provides both best estimates of safe doses of mixtures and confidence limits of those estimates. Lower confidence limits of the estimates of safe doses can be used to evaluate the adequacy of deterministic estimates of safe doses for the mixtures. Finally, the tool identifies components of a mixture that drive the mixture's risk. This information can be used to direct the development of more refined assessments of the mixtures' toxicity or strategies for risk mitigation. The tool can be used to assess mixtures of any number of migrants and including mixtures with migrants having little or no toxicity data. At the same time, the approach allows the use of toxicity information, when available, to improve the assessment of mixtures' toxicity. Two example applications of the tool are provided.

Keywords: probabilistic modelling; risk assessment – modelling; toxicology; food-contact materials; water

Introduction

Food-contact materials (FCMs) release low levels of chemicals (migrants) to foods during transport and storage. Current approaches for the evaluation of the safety of FCMs have evaluated the safety of migrants on a substance-by-substance basis. A single FCM, however, can release multiple migrants to a food. Current approaches leave unanswered the issue of combined effects of multiple chemicals. To address this issue, the current paper develops a quantitative tool for characterizing the chronic non-cancer toxicity of mixtures of migrants.

The tool is based on a combination of existing models of chronic mixture toxicity (Interdepartmental Group on Health Risks from Chemicals (IGHRC) 2009; US Environmental Protection Agency (USEPA) 2000) and two advancements in regulatory toxicology. The first advancement is the use of Cramer classes (Cramer et al. 1978; Munro et al. 1996) that can provide conservative estimates of

compounds' toxicity based on their structure. This use of the Cramer classes is part of a larger programme, the Threshold of Toxicological Concern (Kroes et al. 2004; Barlow 2005). The second advancement is the use of probabilistic models to characterize the uncertainty in derived no effect levels (DNELs). (In this paper, the DNEL is used to refer to a predicted dose that is protective of sensitive humans such as the DNEL (European Chemicals Agency (ECHA) 2008), the reference dose (USEPA 1993), tolerable daily intake, or allowable daily intake.) These two advancements bring different advantages to the assessment of mixture toxicity. The Cramer classes provide a means to characterize conservatively the toxicity of chemicals based on a chemical's structure. Probabilistic models allow the definition of the confidence in the safety associated with a dose of a chemical (Swartout et al. 1998; Carlson-Lynch et al. 1999; Vermeire et al. 2001). The models allow the consideration of the impact of uncertainty in toxicity studies, the use of assessment factors, and the statistical implications of having multiple compounds contribute to the toxicity of mixtures.

The tool presented in this paper addresses chronic non-cancer toxicity. Where one or more of the migrants released by an FCM are suspected of posing carcinogenic risks, a separate assessment of such risks should be performed. The approach does not directly address the issue of synergy between chemicals. However, the proposed tool restricts exposures to very low doses where synergy is not anticipated to occur (Könemann and Pieters 1996).

Materials and Methods

Modelling the chronic non-cancer toxicity of mixtures

Currently, the non-cancer toxicities of mixtures are evaluated using either a dose-additive or an independence model (IGHRC 2009). Dose-additive models assume that each component of a mixture acts jointly by a common mechanism in a manner that is proportional to the compounds' DNELs. Dose-independence models define the risk from mixture as the sum of the independent effects of the components, where each component is viewed as being diluted by the entire mixture. In general, additive models predict higher levels of toxicity for mixtures (lower values for DNELs) than independence models.

Guidance on the selection of which models are appropriate for mixtures has been proposed by a number of groups (USEPA 2000, 2007; Agency for Toxic Substances and Disease Registry (ATSDR) 2004; IGHRC 2009). In general, the independence models are believed to underestimate the toxicity of mixtures since two or more components could produce a joint effect and have an additive response. Additive

models are believed to overestimate toxicity since they assume that all migrants will cause the same effect and their doses will add. By using both models of mixture toxicity, the proposed tool provides bounds to the uncertainty in the toxicity of the mixture that occurs as a result of uncertainty in the degree of additivity between migrants.

Under both the additive and independence models, the safe dose of a mixture is determined based the composition of the mixture and information on the toxicity of each component. The estimate of the safe dose of a mixture under an additive model of mixture toxicity, $mDNEL_A$, is given by:

$$mDNEL_A = \frac{1}{\sum_i^i \frac{F_i}{DNEL_i}} \qquad (1)$$

where F_i is the fraction of the mass of the mixture represented by the *i*th component of the mixture; and $DNEL_i$ is the DNEL for the *i*th component of the mixture (USEPA 2000).

Under the independence model, the safe dose of the mixture is evaluated in terms of the toxicity of each component of the mixture. Thus, setting a safe level for the mixture involves a separate assessment for each mixture component. A dose of a mixture that would only allow a safe dose of the *i*th component of the mixture ($DNEL_i$) is defined as $mDNEL_i$. The value of $mDNEL_i$ for the *i*th component is given by:

$$mDNEL_i = \frac{DNEL_i}{F_i} \qquad (2)$$

The safe dose of a mixture determined by an independence model, $mDNEL_I$, would be the lowest value of $mDNEL_i$ for the mixtures' components (USEPA 2000):

$$mDNEL_I = \min\left(\frac{DNEL_i}{F_i}\right) \qquad (3)$$

The contribution of individual components to an additive model of risk can be evaluated based on the toxicity weight of the component. The toxicity weight is given by the inverse of $DNEL_i$:

$$Toxicity\_weight = \frac{F_i}{DNEL_i} \qquad (4)$$

The component with the largest weight will have the largest impact on $mDNEL_A$ and will determine $mDNEL_I$. The identification of toxicity driver for the mixture can be of great assistance in the management of risks from migrants since it allows the manufacture to focus risk management measures on the migrant that will have the greatest reduction in the mixture's toxicity.

Using the Cramer classes to fill toxicity data gaps

The derivation of $mDNEL_A$ or $mDNEL_I$ for a mixture requires that the assessor have an estimate of the safe dose of each component, $DNEL_i$. In many mixtures, the components may include compounds with little or no toxicity information. In the past such data gaps effectively stopped the modelling of mixture risks and required that the assessor develop the necessary toxicity data on the components or test the mixture as a whole. The Cramer classes approach provides a means of deriving conservative estimate of the $DNEL_i$ for organic chemicals without toxicity information.

Any organic chemical with a known structure can be assigned into one of the three Cramer classes. A conservative estimate of the no observed effect level (NOEL) and $DNEL_i$ for chemicals in each category has been established by Munro et al. (1996) (Table 1).

These values are conservative since 95% of all chemicals in a given Cramer class have $DNEL_i$ values that are greater than the values given in Table 1 and in many instances may have values that exceed these values by orders of magnitude (Munro et al. 1996).

As discussed by Kroes et al. (2004), it is important to determine if the structures of the migrants are represented in the chemicals in the Munro database. If a migrant has a unique structure, the use of Cramer classes may not be appropriate.

Table 1. Values of no observed effect level (NOEL) and predicted safe dose for the three Cramer classes.

Cramer class	NOEL (mg kg^{-1} body weight day^{-1})	Safe dose (mg kg^{-1} body weight day^{-1})
I	3	0.03
II	0.91	0.0091
III	0.15	0.0015

Probabilistic models of non-cancer risks

In the last twelve years, a number of researchers have investigated the uncertainty in non-cancer toxicity and risk assessment using probabilistic models (Price et al. 1995; Baird et al. 1996; Slob and Pieters 1997, 1998; Swartout et al. 1998; Vermeire et al. 1999, 2001; Gaylor and Kodell 2000; Kalberlah et al. 2003; Kodell and Chen 2007). These efforts have sought to organize information on the uncertainties in each step of the process of establishing a DNEL and to quantify the uncertainty in the estimate of the DNEL. The result of these efforts are the creation of probability density functions that describe the probability a dose of a chemical is protective of 'sensitive individuals' in an exposed population given the limitations in the current understanding of toxicology (Slob and Pieters 1997, 1998; Swartout et al. 1998; Gaylor and Kodell 2000). These studies have found that the degree of uncertainty in DNELs varies across chemicals and increases with the uncertainty in measures of a chemical's chronic endpoints and number of assessment factors used in setting a chemical's DNEL. In general, the studies have found that DNELs established using traditional values for assessment factors are protective and have a high likelihood of overestimating the protective dose by an order of magnitude or more. The quantitative information on the uncertainty in the DNEL have been incorporated into models of the uncertainty and variation in non cancer risk findings (Price et al. 1997; Carlson-Lynch et al. 1999; Bosgra et al. 2005; Van der Voet and Slob 2007).

In this project, the probabilistic models used to characterize the uncertainty in the DNELs of individual compounds are extended to predict the uncertainty in the $mDNEL_A$. This step is taken in order to avoid overestimating the toxicity of a mixture. This overestimation can occur because DNELs are lower confidence limits of estimates of dose that are protective for that chemical. In the case of mixtures, using the DNELs for each mixture component results in an overestimation of toxicity since the probability that all components of a mixture are as toxic as their DNELs becomes very small as the number of mixture components increases. This unintentional bias of the estimate of mixture toxicity can be avoided by modelling the uncertainty in the toxicity of the mixture and setting a consistent percentile of the distribution as the basis for the mixture's final toxicity (Carlson- Lynch et al. 1999).

Development of probabilistic models of mixture toxicity

As discussed by Vermeire et al. (2001), the inputs in the equation used to set the DNEL are modelled as uncertain variables. Equation (5) sets the DNEL for the *i*th chemical of a mixture $DNEL_i$:

$$DNEL_i = \frac{POD_i}{\prod AF_{ki}} \qquad (5)$$

where POD_i is the point of departure of the critical effect for the *i*th chemical; and AF_{ki} is the *k*th assessment factor used in setting $DNEL_i$. (Instead of the term 'adjustment', other authors have used 'extrapolation', 'safety', 'assessment', or 'uncertainty' factors for these parameters.) Substituting this definition into Equations (1) and (3) gives:

$$mDNEL_A = \frac{1}{\sum\left(\frac{F_i \prod AF_{ki}}{POD_i}\right)} \qquad (6)$$

$$mDNEL_I = \min\left(\frac{POD_i}{F_i \prod AF_{ki}}\right) \qquad (7)$$

In this analysis, the uncertainty in $mDNEL_A$ and $mDNEL_I$ are determined by Monte Carlo modelling using distributions for the uncertainty in the PODs and uncertainty factors. The analysis is performed using Microsoft Excel™ and Crystal Ball™ (an Excel add-on program for probabilistic analysis). The following sections present the sources of data and approaches used for characterizing the uncertainty in POD_i and the various types of uncertainty factors.

Uncertainty in POD_i

POD_i in the $DNEL_i$ is traditionally the no observed adverse effect level (NOAEL) from a chronic animal study, but for many chemicals it could be the lowest observed adverse effect level (LOAEL), or a benchmark dose, or if toxicity data are not available, the 5th percentile of the compound's Cramer class. The uncertainty in POD_i varies from chemical to chemical and depends on both the mode of action of a chemical and the nature of the study of that defines POD_i.

In this paper, if the POD_i of a chemical is based on a NOAEL or LOAEL, the uncertainty in POD_i is assumed to be described by a triangular distribution where the reported NOAEL or LOAEL dose is the most likely value of POD_i and the maximum and minimum are one-third and three times the most likely values. The selection of this range is based on the observation that the dose spacing in most animal studies is a value of three or less.

When POD_i is derived using the Cramer classes, the uncertainty in POD_i is determined using the distribution of the NOELs associated with each of the Cramer classes. This approach takes advantage of the fact that the distributions of NOAELs

for each of the three Cramer classes are measures of the uncertainty in the POD of a chemical that is conditional on the chemical's Cramer class. The distribution for each Cramer class is developed by sampling from the discrete values of the NOELs in each class as presented in Munro et al. (1996, appendix).

Distributions for assessment factors AF_{ki}

There are a number of uncertainty factors that are used in setting DNELs. These include:

- interspecies uncertainty;
- interindividual uncertainty;
- exposure duration extrapolation;
- database adequacy; and
- LOAEL to NOAEL adjustment.

The number and specific nature of the uncertainty factors used in assessing each component of a mixture are determined by the toxicity data available for the component.

The concept of replacing the single value of an uncertainty factor with a distribution has been described by Brand et al. (1999, 2001) and Swartout et al. (1998). The distributions for each uncertainty factor are based on the inter-chemical variation in the size of the adjustment in dose required to go from one measure of toxicity to another. The interspecies factor is based on going from a POD in animals to a POD in typical humans. The interindividual factor is based on going from a POD for typical humans to a POD in sensitive humans. Similar distributions are set for the subchronic and database uncertainty factors. Estimates of the distributions can be based on policy considerations (Slob and Pieters 1998; Swartout et al. 1998) or empirical data (Bokkers and Slob 2007; Price et al. 2008).

Interspecies assessment factor

The interspecies uncertainty factor has been the subject of a number of publications (Rhomberg and Wolff 1998; Kalberlah et al. 2002; Schneider et al. 2004; Bokkers and Slob 2007; Price et al. 2008).

In the present paper, the distribution for this factor is based on the two-part approach (allometric scaling plus a residual uncertainty term) proposed by Schneider et al. (2004, 2006) and Bokkers and Slob (2007). The data for the

distribution of residues are taken from Schneider et al. (2006). The allometric term is a function of the test animal used in the study that set the NOAEL. Table 2 presents the allometric scaling factors proposed in recent REACH guidance (ECHA 2008) and in Vermeire et al. (2001).

The distribution for the residual uncertainty term is taken from Schneider et al. (2006) who proposed a lognormal distribution with a geometric mean and geometric standard deviation of 0.97 and 3.24, respectively.

Interindividual assessment factor

Data on interindividual variation and the size and variation of the factor required to address it have been qualitatively evaluated (Schneider et al. 2004, 2006). These authors proposed the use of a displaced lognormal distribution with a displacement of 1.00 and a geometric mean and geometric standard deviation of 2.31 and 3.57, respectively.

Exposure duration extrapolation factor

Data on the uncertainty in extrapolating from subchronic to chronic PODs have been investigated by both Bokkers and Slob (2005) and Kalberlah et al. (2003). Kalberlah et al. used ratios of NOAELs to investigate the effect of extrapolating from subchronic to chronic doses. Bokkers and Slob used benchmark doses to investigate the assessment factor. This approach avoids a portion of the 'noise' in the measurements created by the use of NOAELs and thus provides a better estimate of the true distribution. Bokkers and Slob reported that the distribution is lognormal with a geometric mean and geometric standard deviation of 1.7 and 2.9, respectively.

Database adequacy and LOAEL to NOAEL uncertainty factors

There are limited data on the inter-chemical variation in the extrapolation of the LOAEL to NOAEL and the database adequacy factors. In this project, we have used the policy-based distribution proposed by Swartout et al. (1998). If the standards for a migrant use a full factor of 10 then the Swartout distribution is used; if the standards use a partial factor (2 or 3 rather than 10), then the square-root of values from the Swartout distribution are used.

Table 2. Allometric scaling factors for common test species

Mouse	7
Rat	4
Dog	1.4
Monkey	2

Application of the Tool to Experimental Data

This paper presents two example applications of the tool. The first application is the assessment of the toxicity of a mixture of migrants extracted from a sample of polystyrene using ethanol (Bradley and Coulier 2007). The second application is an evaluation of the toxicity of a set of migrants observed in water stored in polypropylene containers (Skjevrak et al. 2005).

Bradley and Coulier (2007) performed a study of a number of FCMs and simulants. The focus of the study is on low-level migrants not intentionally added to FCMs. In a study of polystyrene, seven compounds were identified; five of the compounds had their exact structures identified and the remaining two were identified as a 'branched alkane' and 'benzothiophene related'. Skjevrak et al. (2005) analysed water stored in polypropylene bottles. Water was stored at ambient temperatures for 72 h in test bottles and analysed for migrants. Seven compounds were detected. The migrants' structures and concentrations were reported; Table 3 presents the residue levels and the structures of the compounds detected in the two studies.

As Table 3 indicates, Example 1 expresses the mixture in terms of $\mu g dm^{-2}$ and Example 2 in terms of $\mu g\ 1^{-1}$. In both cases, these values are a measure of potential exposures. The actual exposures are determined by using these values along with information on contact rates (surface area/mass of specific food), food or water intake rates, and body weights. However, the mass fractions of each migrant in the mixture that would be consumed (F_i) will be proportional to the fractions of the measurements of migration rates in Example 1 and the concentrations in Example 2.

Table 3. Migrant levels reported in the two examples

Compound	Extraction rate in ethanol ($\mu g\,dm^{-2}$)	Concentration in drinking water ($\mu g\,l^{-1}$)
Example 1		
2-Ethyl 1-hexanol	3.0	
Phthalic anhydride	1.0	
Branched alkane	1.0	
Benzothiophene related	3.0	
2-(2′-Hydroxy-5′-methylphenyl) benzotriazole	1.0	
2-Diethyl hexyl phthalate	5.0	
Ethylene*bis*(oxyethylene)*bis*-(3-(5-*tert*-butyl-4-hydroxy-*m*-tolyl)-propionate)	1.0	
Example 2		
Di isobutyl phthalate		36
Dibutyl phthalate		9
Ethyl-4-ethoxybenzoate		101
2,4-Di-*tert*-butylphenol		25
Ethylbenzoate		15
4-Methylbenzaldehyde		37

An Internet search for guidance values for oral doses was performed on the 13 compounds. Three of the six migrants in Example 1 and one of the seven migrants in Example 2 had a safe dose established by a regulatory body. Cramer classes were determined using ToxTree-v0.01 (developed in 2005 by Ideaconsult Ltd, Sofia, Bulgaria); eleven of the substances had their structures completely identified and the remaining two compounds were sufficiently defined to allow their assignment to Cramer classes. The 'branched alkane' is assigned to Cramer class I and the 'benzothiophene related' is assigned to Cramer class III.

Table 4 presents the toxicity data and Cramer classes for each of the migrants. It presents the original standard, the POD, and the assessment factors used in setting the original standard. In this paper, the values of the assessment factors were revised to reflect the most recent technical guidance on the values of these factors ECHA (2008). This guidance specifies values that differ from earlier standard setting approaches. The resulting DNELs for the compounds differed by up to a factor of five from the original standards. The final values of the DNELs are presented in the last column of Table 4.

Deterministic and probabilistic version of the additive and independence models of the mixture DNELs were constructed using Equations (1), (3), (6), and (7), respectively. The distributions used were selected based on the assessment factors required for each compound. The probabilistic models were simulated 50 000 times using Latin hypercube sampling. This number of iterations was found to give stable estimates of the 5th, 50th, and 95th percentiles for the mixture toxicity under the two models.

Table 4. Cramer classes and toxicity data for migrants in examples 1 and 2.

Compound	Existing standard (mgkg^{-1} body weight day^{-1})	Source	Cramer class	Test species for POD	Point of departure (mg kg^{-1} body weight day^{-1})	Assessment factors (European Chemicals Agency (ECHA) 2008)					DNEL (mgkg^{-1} day^{-1})
						Inter-species	Intra-species	Exposure duration	LOAEL to NOAEL	Low quality database	
Example 1											
2-Ethyl 1-hexanol	0.5	Joint FAO/WHO (1997)	I	Rat	50	10	10	1	1	1	0.500
Phthalic anhydride	2	USEPA (1988)	III	Mouse	1.562	17.5	10	1	10	1	0.89
Branched alkane			I		3	10	10	1	1	1	0.030
Benzophenone related			III		0.15	10	10	1	1	1	0.0015
2-(2-Hydroxy-5'-methylphenyl) benzotriazole			III		0.15	10	10	1	1	1	0.0015
2-Diethyl hexyl phthalate	0.02	USEPA (2000)	I	Guinea pig	19	3	10	2	3	1	0.11
Ethylene-*bis*(oxyethylene)*bis*-(3-*tert*-but yl-4-hydroxyhydrocinnamate)			I		3	10	10	1	1	1	0.030
Example 2											
Di isobutyl phthalate			I		3	10	10	1	1	1	0.030
Dibutyl phthalate	0.01	AFC (2005)	I	Rat	2	10	10	1	2	1	0.01
Ethyl-4 Ethoxybenzoate			II		0.91	10	10	1	1	1	0.0091
2,4-Di-*tert*-but ylphenol			I		3	10	10	1	1	1	0.030
Ethylbenzoate			I		3	10	10	1	1	1	0.030
4-Methylbenzaldehyde			I		3	10	10	1	1	1	0.030

Finally, the relative weights of each of the mixtures' components were determined using Equation (4).

In Example 2, the data on the levels of the migrants are expressed as $\mu g1^{-1}$. Using the exposure assumptions proposed by Skjevrak et al. (2005), 2 litres of water consumed per day and a 60 kg body weight, it is possible to make a conservative

prediction of the dose of the mixture received by an adult consuming the water. This prediction can be compared against the mixture DNELs developed by our proposed tool.

Results

The results of the analysis are presented in Tables 5 and 6. The toxicities of the two mixtures of migrants are similar under the deterministic additive and independence models. In Example 1, $mDNEL_I$ is 39% larger than $mDNEL_A$; in Example 2, it is 34% larger. These findings suggest that in both mixtures one migrant dominates the toxicity of each mixture. This is confirmed by Table 6, where the migrant with 'benzothiophene related' structure in Example 1 and ethyl-4-ethoxybenzoate in Example 2 have weights that are much larger than any other mixture components.

The probabilistic models of the uncertainty in $mDNEL_I$ and $mDNEL_A$ confirm that the point estimates are conservative. The 5th percentiles of the uncertainty distributions are approximately two- to three-fold higher than the deterministic values. The central values for the $mDNEL_I$ and $mDNEL_A$ are more than 60-fold higher than the deterministic values.

In Example 2, the estimates of mixture toxicity range from 0.014 to 0.043 mg kg^{-1} body weight day^{-1}. The total amount of the mixture in any of the samples was less than 223 µg 1^{-1}. Using the above exposure assumptions, this concentration corresponds to a daily dose of 0.0074 mg kg^{-1} body weight day^{-1}. Since this dose is less than any of the predicted mixture DNELs, the collective toxicity of the migrants are unlikely to pose a risk to individuals consuming water stored in the polypropylene bottles.

Table 5. Estimates of the mixtures' derived no effect levels (DNELs) under assumptions of additivity ($mDNEL_A$) and independence ($mDNEL_I$) (mg kg^{-1} body weight day^{-1}) for examples 1 and 2.

	Example 1		Example 2	
	$mDNEL_A$	$mDNEL_I$	$mDNEL_A$	$mDNEL_I$
Deterministic values	0.0054	0.0075	0.014	0.020
Probabilistic values				
Percentile				
5	0.012	0.013	0.040	0.043
50	0.36	0.50	0.79	1.2
95	3.2	6.2	5.5	11

Discussion

The above findings provide useful guidance to risk managers for the evaluation of risks posed by migrants from FCMs. The approach provides specific estimates of mixture DNELs that can be used to evaluate the risks posed by mixtures of migrants. When the doses of mixtures of migrants are below the mixtures' DNEL values, the assessor can have confidence that the mixture will not pose an unacceptable non-carcinogenic risk. The values of $mDNEL_I$ and $mDNEL_A$ for a mixture can be compared with the uncertainty distributions to determine if the deterministic values are under- or over-protective. In these examples the doses of the mixtures could exceed the deterministic values by roughly a factor of two and still be highly protective.

In addition, the uncertainty distributions demonstrate that the actual toxicity levels for the two example mixtures of migrants are likely to be 60-fold higher than the deterministic values. This finding reflects both the magnitude of the uncertainty in the estimates and the level of precaution built into the assessment approach.

When the exposures to the mixtures exceed the deterministic values of $mDNEL_I$ and $mDNEL_A$ or the 5th percentiles of the probabilistic value of mDNELI and $mDNEL_A$, the risk manger is given guidance on which component should be addressed first. In Example 2, risk managers should focus their efforts on ethyl-4-ethoxybenzoate.

Table 6. Toxicity weightings for migrants in examples 1 and 2.

Compound	Toxicity weights
Example 1	
2-Ethyl 1-hexanol	0.4
Phthalic anhydride	0.1
Branched alkane	2.2
Benzothiophene related	133.3
2-(2′-Hydroxy-5′-methylphenyl) benzotriazole	44.4
2-Diethyl hexyl phthalate	3.2
Ethylene-*bis*(oxyethylene) *bis*-(3-(5-*tert*-butyl-4-hydroxy-*m*-tolyl)-propionate)	2.2
Example 2	
Di-isobutyl phthalate	5.4
Di-butyl phthalate	4.0
Ethyl-4-ethoxybenzoate	49.8
2,4-Di-*tert*-butylphenol	3.7
Ethylbenzoate	2.2
4-Methylbenzaldehyde	5.5

Addressing this risk could involve modifying the FCM to reduce the concentration of the migrant. Alternatively, the risk manager could choose to develop toxicity estimates for the compound since the toxicity of ethyl-4-ethoxybenzoate is established using Cramer classes. If actual data were available for the compound there is a large probability that the POD would be larger than the value used in this assessment (0.91 mg kg^{-1} body weight day^{-1}). Replacing the value of the POD used in this assessment with actual data is therefore likely to lower the prediction of the mixtures risk.

Conclusions

This project has demonstrated that a combination of existing mixture models, the use of Cramer classes to estimate toxicity, and probabilistic modelling of the uncertainty in estimates of safe levels of exposure can provide useful characterizations of the toxicity of mixtures. The proposed tool has the ability to:

- estimate the toxicity of mixtures composed of any number of migrants;
- estimate the toxicity of mixtures that include certain organic chemicals with little or no toxicity data;
- allow the use of toxicity information on individual chemicals (when it is available) to improve the assessment of mixture toxicity; and
- quantify the uncertainty in the predicted safe doses.

However, the approach produces estimates that are biased towards overestimating toxicity. As a result, the approach will be most useful in screening out low-risk mixtures. The technique also provides guidance on which migrants warrant the greatest attention.

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References

AFC. 2005 Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Material in Contact with Food (AFC) on a request from the Commission related to di-butylphthalate (DBP) for use in food-contact materials Question No. EFSA-Q-2003-192 Adopted on 23 June 2005 by written procedure. EFSA J P. 242.

Agency for Toxic Substances and Disease Registry (ATSDR). 2004. Guidance manual for the assessment of joint toxic actions of chemical mixtures. Atlanta (GA): US Department of Health and Human Services, Public Health Service.

Baird SJS, et al. 1996. Noncancer risk assessment: a probabilistic alternative to current practice. Hum Ecol Risk Assess. 2(1):79–102.

Barlow S. 2005. Threshold of toxicological concern (TTC) – A tool for assessing substances of unknown toxicity present at low levels in the diet. Washington (DC): International Life Sciences Institute.
Bokkers BG, Slob W. 2005. A comparison of ratio distributions based on the NOAEL and the benchmark approach for subchronic-to-chronic extrapolation. Toxicol Sci. 85(2):1033–1040.
Bokkers BG, Slob W. 2007. Deriving a data-based interspecies assessment factor using the NOAEL and the benchmark dose approach. Crit Rev Toxicol. 37(5):355–373.
Bosgra S, Pos PMJ, Vermeire TG, Luit RJ, Slob W. 2005.Probabilistic risk characterization: an example with di(2-ethylhexyl) phthalate. Regul Toxicol Pharmacol. 43(1):104–113.
Bradley E, Coulier L. 2007. An investigation into the reaction and breakdown products from starting substances used to produce food contact plastics. York (UK): Central Science Laboratory.
Brand KP, Catalano PJ, Hammitt JK, Rhomberg L, Evans JS. 2001. Limitations to empirical extrapolation studies: the case of BMD ratios. Risk Anal. 21(4):625–640.
Brand KP, Rhomberg L, Evans JS. 1999. Estimating noncancer uncertainty factors: are ratios NOAELs informative? Risk Anal. 19(2):295–308.
Carlson-Lynch H, Cohen JT, Graham JD, Shlyakhter AI, Evans JS. 1999. Application of quantitative information on the uncertainty in the RfD to noncarcinogenic risk assessments. Hum Ecol Risk Assess. 5(3):527–546.
Cramer GM, Ford RA, Hall RL. 1978. Estimation of toxic hazard – a decision tree approach. Food Cosmet Toxicol. 16(3):255–276.
European Chemicals Agency (ECHA). 2008. Characterisation of dose [concentration]–response for human health. In: Guidance on information requirements and chemical safety assessment. Helsinki (Finland): ECHA.
FAO/WHO 1997. Joint FAO/WHO Expert Committee on Food Additives 2-ETHYL-1-HEXANOL FAS 40-JECFA 49/189. Available from: http://www.inchem.org/ documents/jecfa/jeceval/jec763.htm
Gaylor DW, Kodell RL. 2000. Percentiles of the product of uncertainty factors for establishing probabilistic reference doses. Risk Anal. 20(2):245–250.
Interdepartmental Group on Health Risks from Chemicals (IGHRC). 2009. Chemical mixtures: A framework for assessing risks (CR14). Cranfield (UK): Institute of Environment and Health, Cranfield University.
Kalberlah F, Fost U, Schneider K. 2002. Time extrapolation and interspecies extrapolation for locally acting substances in case of limited toxicological data. Ann Occupat Hyg. 46(2):175–185.
Kalberlah F, Schneider K, Schuhmacher-Wolz U. 2003. Uncertainty in toxicological risk assessment for noncarcinogenic health effects. Regul Toxicol Pharmacol. 37(1):92–104.
Kodell RL, Chen JJ. 2007. On the use of hierarchical probabilistic models for characterizing and managing uncertainty in risk/safety assessment. Risk Anal. 27(2):433–437.
Könemann WH, Pieters MN. 1996. Confusion of concepts in mixture toxicology. Food Chem Toxicol. 34(11–12):1025–1031.
Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, Wurtzen G. 2004. Structure based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem Toxicol. 42(1):65–83.
Munro IC, Ford RA, Kennepohl E, Sprenger JG. 1996. Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern. Food Chem Toxicol. 34(9):829–867.
Price PS, Keenan RE, Swartout JC, Gillis CA, Dourson MC. 1995. A Monte Carlo based approach for modelling noncarcinogenic dose response rates. Paper presented at: Society for Risk Analysis Annual Meeting, 3–5 December 1995; Honolulu, HI, USA.
Price PS, Keenan RE, Swartout JC, Gillis CA, Carlson-Lynch H, Dourson ML. 1997. An approach for modelling noncancer dose responses with an emphasis on uncertainty. Risk Anal. 17(4):427–437.
Price PS, Keenan RE, Swartout JC. 2008. Characterizing interspecies uncertainty using data from studies of anti-neoplastic agents in animals and humans. Toxicol Appl Pharmacol. 233(1):64–70.

Rhomberg LR, Wolff SK. 1998. Empirical scaling of single oral lethal doses across mammalian species based on a large database. Risk Anal. 18(6):741–753.
Schneider K, Schuhmacher-Wolz U, Hassauer M, Darschnik S, Elmha¨ user E, Mosbach-Schulz O. 2006. A probabilistic effect assessment model for hazardous substances at the workplace. Regul Toxicol Pharmacol. 44(2):172–181.
Schneider K, Oltmanns J, Hassauer M. 2004. Allometric principles for interspecies extrapolation in toxicological risk assessment – empirical investigations. Regul Toxicol Pharmacol. 39(3):334–347.
Skjevrak I, Brede C, Steffensen I, Mikalsen A, Alexander J, Fjeldal P, Herikstad H. 2005. Non-targeted multi-component analytical surveillance of plastic food-contact materials: Identification of substances not included in EU positive lists and their risk assessment. Food Addit Contam. 22(10):1012–1022.
Slob W, Pieters MN. 1997. A probabilistic approach for deriving acceptable human intake limits and human health risks from toxicological studies: General framework. Bilthoven (the Netherlands): RIVM. Report Number 620110005.
Slob W, Pieters MN. 1998. A probabilistic approach for deriving acceptable human intake limits and human health risks from toxicological studies: General framework. Risk Anal. 18(6):787–798.
Swartout JC, Price PS, Dourson DL, Carlson-Lynch H, Keenan RE. 1998. A probabilistic framework for the reference dose (probabilistic RfD). Risk Anal. 18(3):271–282.
US Environmental Protection Agency (USEPA). 1988. US EPA Integrated Risk Information System ‘‘Phthalic Anhydride’’. Available from: http://www.epa.gov/ncea/iris/subst/0308.htm
US Environmental Protection Agency (USEPA). 1993. Reference dose (RfD): Description and use in health risk assessments: Background Document 1A [Internet]. Washington (DC): USEPA, Office of Research and Development, National Center for Environmental Assessment; [cited 2008 Dec 1]. Available from: http://www.epa.gov/IRIS/rfd.htm/
US Environmental Protection Agency (USEPA). 2000. Supplementary guidance for conducting health risk assessment of chemical mixtures. In: Risk assessment forum. Washington (DC): USEPA, Office of Research and Development. EPA Number 630/R-00-002.
US Environmental Protection Agency (USEPA). 2007. Concepts, methods and data sources for cumulative health risk assessment of multiple chemicals, exposures and effects: A resource document. Cincinnati (OH): USEPA, Office of Research and Development, National Center for Environmental Assessment. EPA Number 600/R-06/013F.
Van der Voet H, Slob W. 2007. Integration of probabilistic exposure assessment and probabilistic hazard characterization. Risk Anal. 27(2):351–371.
Vermeire T, Stevenson H, Pieters MN, Rennen M, Slob W, Hakkert BC. 1999. Assessment factors for human health risk assessment: a discussion paper. Crit Rev Toxicol. 29(5):439–490.

Chapter 7

Maximum cumulative ratio (MCR) as a tool for assessing the value of performing a cumulative risk assessment

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Abstract

Due to the vast number of possible combinations of chemicals to which individuals are exposed and the resource-intensive nature of cumulative risk assessments, there is a need to determine when cumulative assessments are most required. This paper proposes the use of the maximum cumulative ratio (MCR) as a tool for this evaluation. MCR is the ratio of the cumulative toxicity received by an individual from exposure to multiple chemical stressors to the largest toxicity from a single chemical stressor. The MCR is a quantitative measure of the difference in an individual's toxicity estimated using a chemical-by-chemical approach and using an additive model of toxicity. As such, it provides a conservative estimate of the degree to which individuals' toxicities could be underestimated by not performing a cumulative risk assessment. In an example application, MCR is shown to be applicable to the evaluation of cumulative exposures involving up to 81 compounds and to provide key insights into the cumulative effects posed by exposures to multiple chemicals. In this example, MCR values suggest that individuals exposed to combinations of chemicals with the largest Hazard Indices were dominated by the contributions of one or two compounds.

Keywords: cumulative; risk; exposure; mixtures

Introduction

The Concern for Cumulative Toxicity from Concurrent Exposure to Multiple Chemicals

Humans are constantly exposed to multiple chemicals from multiple sources [1-4]. However, regulatory programs such as TSCA in the United States and REACH in the European Union evaluate risks on a chemical-by-chemical basis and do not require the consideration of cumulative exposures when determining human health effects. It has been asserted that the determination of toxicity on this basis could underestimate the total toxicity to individuals [4]. The chemical-by-chemical approach is believed to underestimate toxicity when the combined exposures to chemicals result in a cumulative toxicity that exceeds the toxicity of the most toxic of the individual chemicals. In these instances, a chemical-by-chemical approach could find that each chemical posed no unacceptable risk, but the mixture of chemicals could result in unacceptable effects.

The difficulty with applying these approaches to aggregate or cumulative exposures is that an individual who receives high levels of exposure from one

source will not necessarily receive high levels of exposure from a second or a third source.

Tools for evaluating risk from cumulative exposures have been developed by the U.S. Environmental Protection Agency [3], and other organizations [5-7]. Tiered approaches for evaluation of cumulative exposures have also been developed by the World Health Organization (WHO) [6,7]. However, there has been relatively little investigation into the magnitude of the toxicity missed if a cumulative risk assessment is not performed. This paper addresses this gap and is intended to be fully compatible with the WHO framework.

The maximum cumulative ratio

In this paper we describe a simple tool, the Maximum Cumulative Ratio (MCR) that provides a quantitative measure of the magnitude of the toxicity that is underestimated by not performing a cumulative risk assessment. The MCR is defined as the ratio of the toxicity received by an individual from exposures to multiple chemicals (cumulative toxicity) to the largest toxicity received by the individual from any one chemical (maximum chemical toxicity).

This paper applies the concept of the MCR to estimates of toxicity derived from dose additive models. Dose additive models include simple conservative screening approaches that do not consider mechanism of action or the target organs (WHO Tier 1 assessments) and more refined assessments that do consider these factors (WHO Tier 2 assessments). Under additive models, a risk ratio is created by dividing the dose of an individual chemical by a measure of the chemical's toxicity. In the case of the Hazard Index approach [4], this measure is the "permitted" dose for the chemical. Permitted doses include regulatory standards and guidance values such as the Reference Dose, Population Adjusted Dose, Allowable Daily Dose, Tolerated Daily Intake, or Derived No Effect Level. Other additive models of toxicity include the Toxicity Unit approach used in aquatic toxicology [3,8].

The values of MCR for individuals in a population (MCR_i) determined using the Hazard Index (HI) approach is calculated using the following equations. The measure of cumulative toxicity received by the i^{th} individual in a population exposed to *n* chemicals is given by the individual's hazard index (HI_i):

$$HI_i = \sum^{n} HQ_{ij} \qquad (1)$$

where HQ_{ji} is the hazard quotient contributed from the dose of the j^{th} of the *n* chemicals to the i^{th} individual (D_{ij}). The value of HQ_{ji} is given by:

$$HQ_{ij} = \frac{D_{ij}}{PD_j} \quad (2)$$

PD_j is the permitted dose of the j^{th} chemical for humans. The maximum of the chemical-specific toxicities for the i^{th} individual is given by:

$$MHQ_i = Max(HQ_i) \quad (3)$$

The value of MCR for the i^{th} individual ($MCR_{i,}$) is given by:

$$MCR_i = \frac{HI_i}{MHQ_i} \quad (4)$$

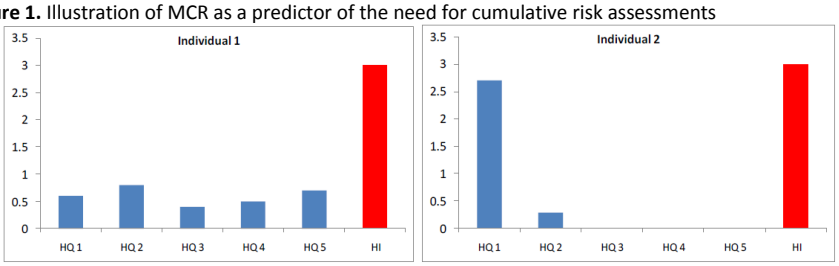
Recognition of the importance of the ratio of the cumulative toxicity to the maximum toxicity from any one chemical in assessing the toxicity of mixtures is not new. The ratio has been used in the field of aquatic toxicology in the evaluation of the effects of mixtures. In 1981, Konemann [8] proposed the use of this ratio as part of a quantitative strategy for the determination of the type of joint action of mixtures of chemicals for fish. Konemann noted that the range of the ratio is bounded by 1 and n, where *n* is the number of chemicals in a mixture. The ratio has a value of 1 for mixtures where all of the mixture's toxicity comes from one component. A mixture will have a value of *n* when all the chemicals are present in equitoxic doses.

Junghans *et al.* [9] observed that the ratio could be used to predict when dose additive and independent action models of a mixture's toxicity produce similar or divergent estimates of toxicity. When ratio values for a mixture are close to 1, the dose additive and independent action models produce virtually identical results. The authors go on to note that the toxicities of mixtures with ratios close to 1 are dominated by the contributions of a few components.

The concept of MCR builds on Junghans *et al.*'s observation that the ratio is a measure of whether individuals' cumulative exposures are dominated by a single chemical or are the result of the contribution of many chemicals [9]. As the illustration in Figure 1 demonstrates, dominance of one chemical is a critical factor in determining the need for a cumulative risk assessment. In this illustration, two individuals are assumed to have cumulative exposures to five chemicals. The hazard indices of the two individuals are 3. For the first individual the values of the Hazard Quotients for the five chemicals are, 0.6, 0.8, 0.4, 0.5, and 0.7. For the

second individual the values are 2.7, 0.29, 0.008, 0.001, and 0.001 (Figure 1). For the first individual no single chemical is a concern (all Hazard Quotients are less than 1.0), yet the cumulative measure of toxicity is 3 times the level of concern. Thus, a cumulative risk assessment is necessary for individual 1. For the second individual, a chemical-by-chemical based approach reaches the same conclusion as a cumulative risk assessment—the exposures of the second individual are unacceptable. Thus there is less value in performing a cumulative risk assessment in the second case.

Figure 1. Illustration of MCR as a predictor of the need for cumulative risk assessments



The values of MCR for the two individuals are different. The value of *MCR* for the first individual is 3.8. The value of *MCR* for the second is 1.1. This suggests that values of MCR that are close to 1 indicate a lower need for a cumulative risk assessment. This property of the MCR indicates that the measure can be used to rank the relative importance of performing cumulative risk assessments for different groups of chemicals and different exposed populations.

In addition, MCR can be used as a quantitative estimate of the toxicity missed a cumulative risk assessment is not performed. The estimate of maximum hazard to an individual that can be identified under a chemical-by-chemical approach is MHQ_i. The estimate of the maximum toxicity identified under a cumulative risk assessment (assuming additivity) is HI_i. MCR is the ratio of HI_i to MHQ_i and therefore the fraction of the toxicity that is missed in the i^{th} individual by not performing a cumulative risk assessment is:

$$\text{Missed toxicity}_i = 1 - \frac{1}{\text{MCR}_i} \qquad (5)$$

For example, an MCR value of 2 indicates that 50% of an individual's hazard index would be missed if a chemical-by-chemical method is used to assess the individual instead of a cumulative risk assessment. For a mixture with an MCR value of 1.25, the missing portion is 20%.

Application of the maximum cumulative ratio to mixtures of pest protection products in surface waters of the U.S.

In this example, MCR values are determined for cumulative exposures to multiple pest protection products (PPPs) and degradation products of PPPs measured in surface water samples collected under the National Water-Quality Assessment (NAWQA) program [10]. The cumulative risk analysis performed on the mixtures follows the Tier 1 approach described in the WHO guidance for the assessment of mixtures [7]. In a Tier 1 assessment, the effects of all components are assumed to be addressed by an additive model of toxicity. (Under WHO guidance, mechanism of action is evaluated in Tier 2 assessments.)

Exposures are characterized using a generic exposure scenario that is based on conservative exposure assumptions. The scenario assumes that the levels of chemicals observed in the samples occur in drinking water supplies. The doses of each chemical in the mixture are estimated by assuming that the water is consumed at a rate of 2 litres per day by an adult who weighs 60 kilograms. The permitted doses of the chemicals, PD_j, are based on chronic non-cancer standards for the chemicals. Although the measured data are only a snapshot of levels of chemicals at one point in time, the levels of the chemicals are assumed to be constant over time, thus allowing the use of the chronic standards. Note: The long-term average levels at the sampling locations would result in a smaller range of concentrations of chemicals. (Day-to-day variation would be averaged out.) In particular, the upper bound values will be lower for the long-term averages than for the grab samples. Thus use of chronic standards for the values of PD_j will tend to overestimate the values of HQ_i for samples with high concentrations of PPPs.

The values of MCR and HI from an individual's exposure to the chemicals in each of the samples are determined. These data are used to investigate the relationships between MCR, HI, and the number of chemicals in each mixture (*n*). The questions that are investigated are:

- Do MCR values vary across the samples, and if so, what is the range of MCR values?
- Are the values of MCR closer to *n* or 1?
- Are the MCR values correlated with *n*?
- Are the MCR values correlated with the values of HI_i for the individuals exposed to the chemicals in the samples?
- How does the presence of chemicals that occur at levels below the detection limits affect the determination of the values of HI and MCR associated with exposure to a mixture?

The relationships between HI and MCR are investigated since the MCR values that are of most interest to regulators are those that come from the mixtures of higher toxicity. The relationship between MCR and *n* is investigated since it could provide insights to the values of MCR for complex mixtures where *n* is very large. The impact of non-detects is investigated since non-detects are a significant source of uncertainty in cumulative risk assessments [11].

Experimental Section

Materials

The NAQWA is a program operated by the U.S. Geological Survey and is the first U.S. survey of PPPs and their degradation products performed on a national scale in the U.S. [10]. The NAWQA dataset was chosen for several reasons. First, it is a publically available dataset that includes a large number of samples from a wide range of locations. Second each sample was analyzed for a large number of chemicals. Finally, permitted doses for chronic exposures, PD_j, are available for virtually all of the chemicals analyzed for in the samples.
Data on chemical levels in samples of surface water collected under the NAQWA survey are available from the U.S. Geological Survey's internet site. The dataset can be downloaded at [12]. These data used here were collected over the first decade of the monitoring program (4,380 samples from 1992–2001) and reflect agricultural practices of that period. The number of analytes measured in each of the samples varies by date and location and range from 12 to 81. The number of chemicals detected in the samples ranged from 0 to 29. In total, 83 chemicals were analyzed in one or more samples.

Table 1 presents values of PD_j for 81 of the 83 chemicals. The values of PD_j are largely composed of chronic Reference Doses and chronic Population Adjusted Doses established by the Office of Pesticide Programs of the U.S. Environmental Protection Agency (Table 2). These criteria are based on multiple endpoints and target tissues; however as discussed above, in a Tier 1 assessment the effects are assumed to be additive. Permitted doses were not identified for two chemicals (Fenuron and Neburon). Because of the absence of PD values for these chemicals, they were not included in the cumulative assessment. Omitting these chemicals could result in lower estimates of cumulative risk; however, the frequencies of detection of the compounds in the samples are low (0.2% and 0.1% respectively). Because the compounds rarely occur, omitting the compounds is unlikely to change the general findings for the cumulative exposures to the mixtures.

Table 1. Chronic toxicity standards for chemicals measured in surface water samples

Chemical	Permitted Dose mg/kg/day	Source code[a]	Chemical	Permitted Dose mg/kg/day	Source code	Chemical	Permitted Dose mg/kg/day	Source code
2,4,5-T	0.01	1	Cyanazine	0.00026	5	Molinate	0.001	3
2,4,5-TP	0.008	1	Dacthal	0.01	2	Napropamide	0.12	2
2,4-D	0.005	2	Dacthal monoacid	0.01	2	Norflurazon	0.015	2
2,4-DB	0.03	2	Diethyl atrazine	0.0018	2[b]	Oryzalin	0.12	2
2,6 Diethylaniline	0.006	3[b]	Diazinon	0.0002	2	Oxamyl	0.001	2
3-Hydroxycarbofuran	0.00006	2[b]	Dicamba	0.45	2	*p,p'*-DDE	0.0005	3
Acetochlor	0.02	3	Dichlobenil	0.015	2	Parathion	0.006	7
Acifluorfen	0.004	2	Dichlorprop	0.036	2	Parathion-methyl	0.00002	2
Alachlor	0.01	2	Dieldrin	0.00005	6	Pebulate	0.0007	2
Aldicarb	0.00027	3	Dinoseb	0.001	1	Pendimethalin	0.1	2
Aldicarb sulfone	0.00027	3	Dinitro-*o*-cresol	0.004	6	Phorate	0.00017	2
Aldicarb sulfoxide	0.00027	3	Disulfoton	0.00013	2	Picloram	0.2	2
alpha-HCH	0.008	3	Diuron	0.003	2	Prometon	0.05	2
Atrazine	0.0019	2	EPTC	0.0025	2	Pronamide	0.027	2
Azinphos-methyl	0.00149	2	Ethalfluralin	0.04	2	Propachlor	0.054	2
Benfluralin	0.005	2	Ethoprop	0.0001	2	Propanil	0.009	2
Bentazon	0.03	2	Fluometuron	0.005	2	Propargite	0.04	2
Bromacil	0.1	2	Fonofos	0.002	2	Propham	0.02	1
Bromoxynil	0.015	2	γ-HCH	0.0003	1	Propoxur	0.005	2
Butylate	0.05	2	Linuron	0.0077	2	Simazine	0.0018	2
Carbaryl	0.01	2	Malathion	0.07	2	Tebuthiuron	0.07	2
Carbofuran	0.00006	2	MCPA	0.0044	2	Terbacil	0.013	2
Chloramben methyl ester	0.014	4	MCPB	0.015	2	Terbufos	0.00005	2
Chlorothalonil	0.02	2	Methiocarb	0.005	2	Thiobencarb	0.01	2
Chlorpyrifos	0.00003	2	Methomyl	0.008	2	Triallate	0.025	2
cis-Permethrin	0.25	2	Metolachlor	0.1	2	Triclopyr	0.05	2
Clopyralid	0.15	3	Metribuzin	0.013	2	Trifluralin	0.024	2

[a] See Table 2; [b] PPP metabolites are assumed to have equal toxicity to the parent compound on a molar basis.

Table 2. Sources of toxicity data cited in Table 1

Source Code from Table 1	Source of toxicity data (PD_i)
1	USEPA Integrated Risk Information System . http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction = iris.showSubstanceList.
2	USEPA Office of Pesticide Programs Pesticide Reregistration Status. http://www.epa.gov/opp00001/reregistration/status.htm
3	Regulations.gov. http://www.regulations.gov/#!home
4	http://www.consumersunion.org/pdf/fqpa/ReportCard_appendix1.pdf
5	Minnesota Department of Health. Health Risk Limits for Groundwater 2008 Rule Revision Health Risk Assessment Unit, Environmental Health Division. http://www.health.state.mn.us/divs/eh/risk/guidance/gw/cyanazine.pdf
6	Agency for Toxic Substances and Disease Registry. Toxicological Profiles http://www.atsdr.cdc.gov/ToxProfiles/tp1.pdf
7	USEPA Drinking Water Standards and Health Advisories Table. http://www.epa.gov/region9/water/drinking/files/DWSHATv09.pdf

Preliminary analyses of survey data and development of a subset of mixture samples

The NAWQA dataset includes samples with no detectable levels of chemicals or with only one or two detections. The goal of the assessment is to investigate cumulative risks for individuals exposed to a number of chemicals. In order to obtain a dataset where exposure to a significant number of chemicals occurs, water samples with detectable levels of less than five chemicals were removed from the dataset.

In the NAWQA samples, there are a large numbers of analytes with levels below the detection limits (non-detects). This presents a challenge for characterizing cumulative exposures using monitoring data. While, assessors should not assume that non-detected compounds are absent from samples [11], in large numbers non-detects can drive the estimates of the toxicity of the mixture and the values of MCR. In order to investigate the impact of non-detects on HI and MCR values, the data was analyzed using two assumptions, Case 1 where non-detects are set to zero, and Case 2 where non-detects are assumed to have concentrations equal to the detection limit (DL) divided by the square root of two ($DL/2^{0.5}$). Better methods for estimating the impacts of non-detects are available; however, since the method used here does not have a significant impact on results of samples of greater human health concern (see the "Results and Discussion" Section) this approach is deemed to be sufficient. The assumption of chemical being present at the $DL/2^{0.5}$ is a method frequently used for estimating non-detects [11].

Statistical analyses

Trends in the relationships between HI, MCR, and *n* were evaluated by plotting the data in Microsoft Excel spreadsheet and performing statistical analyses in JMP 8.0.2 (SAS Institute Inc.). Nonparametric correlations between HI, MCR, and *n* were performed using Kendall's rank correlation test. Correlations were evaluated using data on values of individual samples and the medians values for samples that are grouped based on *n*. Medians were not determined for groups with less than 10 samples. Wilcoxon Test (a nonparametric test) was used to compare the MCR values of samples of HI values less than 1 with samples of HI values greater than 1.

Results and Discussion

Results

The final dataset consists of 3,099 samples. Data on the compositions of the mixtures are given in Table 3. As would be expected in a survey analyzing for large numbers of chemicals, there are more non-detects than detects in the samples.

Table 3. The number of chemicals (*n*) in the final set of samples

	Minimum Number	Maximum Number	Average Number
Detected Chemicals	5	29	9
Non-Detects	20	76	61
Number of Chemicals Analyzed for in a Sample	31	81	70

Table 4 presents the values of MCR and HI for Cases 1 and 2. Values of HI ranged over seven orders of magnitude. The inclusion of the contributions of the non-detects had a significant impact on the minimum and mean values of the MCR and HI but not on the maximum values of HI. The number of samples with HI values greater than 1 was similar for the two cases, 63 for Case 1 and 66 for Case 2. Table 5 give the means and ranges of MCR value for samples with HI values above and below 1. MCR values in samples with HI values less than 1 were two fold higher when non-detects were considered but largely unchanged for the samples with HI values greater than 1. These findings suggest that in this dataset non-detects are not an important factor in the determination of values of HI and MCR for samples with values of HI greater than 1.

Table 4. Values of HI and MCR for samples in the final dataset

	HI Values for Samples			MCR Values for Samples		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Case 1 Non-Detects = 0	0.00001	57	0.14	1.0002	4.0	1.8
Case 2 Non-Detects = $DL/2^{0.5}$	0.014	57	0.19	1.001	7.5	4.0

Table 5. Comparison of MCR values of samples with HI greater or less than 1

HI cutoff	HI < 1			HI > 1		
Statistics	Minimum	Maximum	Average	Minimum	Maximum	Average
Case 1	1.002	4.0	1.8	1.0002	3.1	1.3
Case 2	1.024	7.5	4.1	1.0014	3.2	1.3

Figures 2 and 3 present plots of the range of HI values in samples grouped based on *n*. The distributions of samples by *n* are very different in the two cases. In Case 1 the value of *n* is equal to the number of detected chemicals while in Case 2 *n* is equal to the number of analytes. In the NAQWA dataset samples were taken over a 10 year period using a variety of analytical methods; however, most samples were analyzed using variation on one of two analytical methodologies. These two methodologies tested for 48 and 80 analytes [10]. As a result the numbers of analytes in the samples tend to cluster around values of 48 and 80. In contrast the number of detections is more evenly distributed.

Figure 2. The relationship between HI and *n* for Case 1. HI and *n* have a strong positive correlation, Kendall's tau-b value of 0.44 ($p < 0.0001$) based on all samples and Kendall tau-b value of 0.96 ($p < 0.001$) for medians of grouped samples.

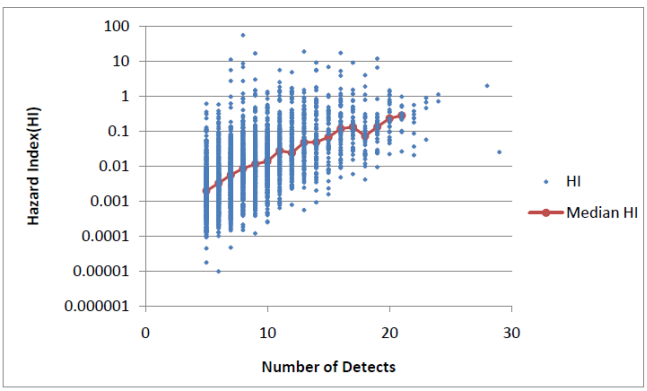
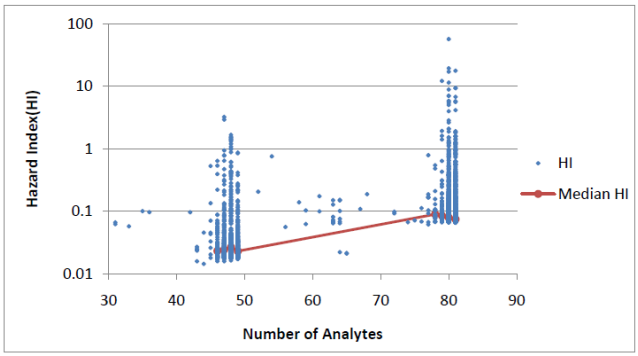


Figure 3. The relationship between HI and *n* for Case 2. HI and *n* have a weakly positive correlation, Kendall's tau-b value of 0.34 ($p < 0.0001$) based on all samples. Correlation based on medians of grouped samples is not statistically significant ($p > 0.05$).



For Case 1, HI was found to be positively correlated with *n* based on analyses of both sample values and the medians for the grouped samples ($p < 0.0001$). The median values of HI were 100-fold larger in samples with 20 detected chemicals than in samples with five detected compounds. For Case 2, HI was weakly correlated with *n* based on sample values and was not statistically correlated with *n* based on median HI values of the grouped samples. These analyses suggest that when *n* is based on the number of detected chemicals (Case 1) it is a strong predictor of the value of HI. When *n* is based on the number of analytes (Case 2) the correlation is much weaker.

Figure 4. The relationship between MCR and *n* for Case 1. MCR and *n* have a weakly positive correlation, Kendall's tau-b value of 0.08 ($p < 0.0001$) based on all samples and Kendall tau-b value of 0.55 ($p < 0.01$) for medians of grouped samples.

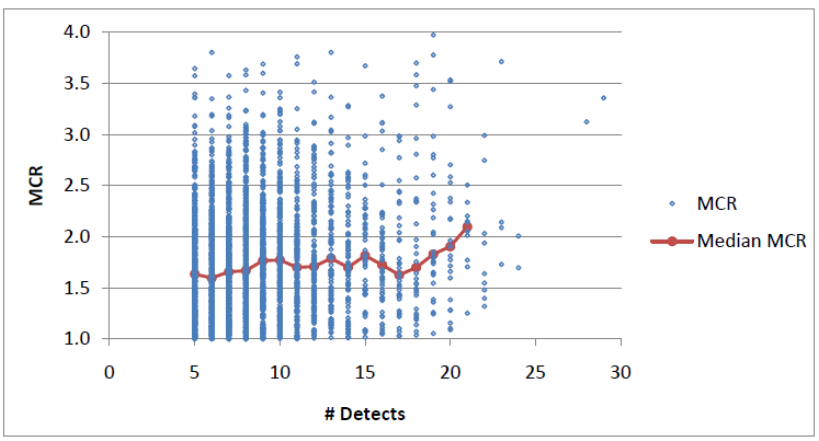
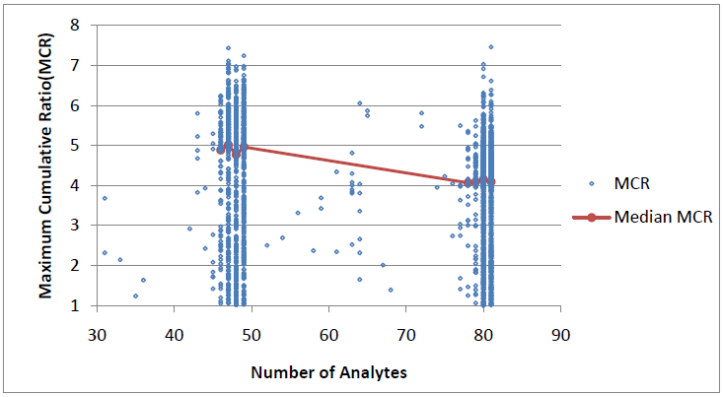


Figure 5. The relationship between MCR and *n* for Case 2. MCR and *n* have a weakly negative correlation, Kendall's tau-b value of –0.12 ($p < 0.0001$) based on all samples. Correlation based on medians of grouped samples is not statistically significant ($p > 0.05$).



Figures 4 and 5 present plots of the range of MCR values as a function of *n* for Cases 1 and 2. When based on data of all individual samples, the correlations between MCR and *n* are weakly positive for Case 1 and weekly negative for Case 2. When based on median MCR values of groups, MCR was found to be weakly correlated with the number of detects (Figure 4) but not the number of analytes (Figure 5). These findings indicate that in this dataset, *n* was not a strong predictor of MCR values in either Case 1 or 2.

Figure 6 presents a plot of MCR *versus* HI for Cases 1 and 2 for all the samples in the final dataset while Figure 7 presents samples with values of HI larger than 1. In Figure 6, the relationship between MCR and HI appears very different for Case 1 and 2. In Case 1, the plot of the MCR samples appear as a diffuse cloud that tapers off at higher value of HI. In contrast, MCR values in Case 2 rise sharply as HI value decreases and separate into two distinct peaks. Additional analysis of the data indicated that the data points in the two peaks came from samples analyzed using the two different analytical methodologies. The left peak came from samples analyzed using the methodology that detected 48 chemicals and the right peak from the methodology that detected 80 samples. As Figure 7 indicates, the impact of the non-detects on the MCR and HI is minimal for samples that have HI values greater than 1.

Results from the Wilcoxon Test showed that the MCR values of samples with HI greater than 1 are significantly lower than samples with HI values less than 1. These differences occurred for both Case 1 and 2 (see Table 2). This suggests that

MCR values are inversely correlated for higher values of HI. Figures 6 and 7 provide additional evidence for this relationship. Tests of the correlation found statistically significant negative correlations between MCR and HI for the entire dataset and for samples with HI values greater than 1. These finding occurred for both Case 1 and 2. For the samples with HI values greater than 1, the MCR values average 1.26 for Case 1 and 1.31 for Case 2. These values of MCR imply that on average 20–25% of cumulative toxicity predicted using the Tier 1 screening models is missed by not performing a cumulative risk assessment on the mixtures in the samples.

Figure 6. Plot of MCR and HI values for all samples. HI and MCR are negatively correlated, Kendall's tau-b values of –0.16 ($p < 0.001$) for Case 1 and values of –0.18 ($p < 0.0001$) for Case 2.

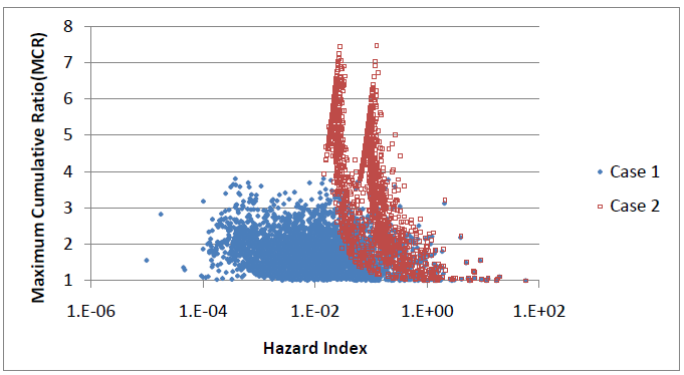
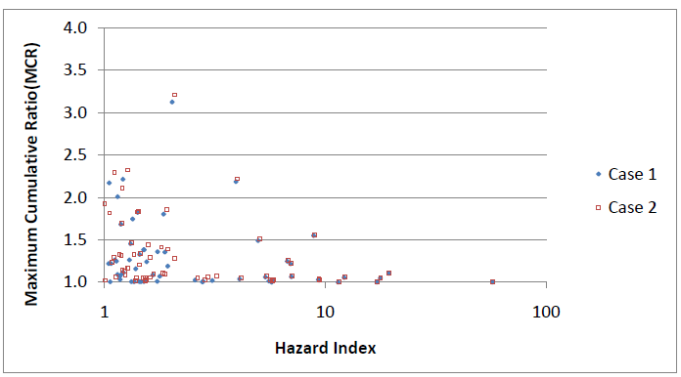


Figure 7. Plot of MCR and HI values for samples with HI greater than 1. HI and MCR are negatively correlated, Kendall's tau-b values of –0.22 ($p < 0.001$) for Case 1 and values of –0.30 ($p < 0.0001$) for Case 2



Discussion

The application of the MCR to the cumulative assessment of the risks from chemicals measured in the NAQWA Dataset demonstrates the potential value of the MCR for characterizing the need for cumulative risk assessments. MCR values were determined for the cumulative toxicities of the chemical mixtures in the 3,099 surface water samples. The values of *n* in the NAQWA samples ranged from 5 to 81. This demonstrates that the MCR approach can be applied to large numbers of cumulative exposures and can be applied to relatively complex mixtures when toxicity data are available for the mixture's components. Non-detects are a significant issue in the analysis of the NAQWA data. Including contributions from non-detects had a significant impact on the estimates of the values of HI and MCR. However, this impact was limited to those samples predicted to have low cumulative toxicity (HI values less than 1).

The values of HI for the samples ranged over five orders of magnitude. The vast majority of the samples (98%) had HI values less than one. Values of MCR range from 1.0–4.0 (mean of 1.8) in Case 1 and 1.0–7.5 (mean of 4.0) in Case 2. These values are much lower than the values of *n* for the samples (31–81 analytes). This indicates that the toxicities of all of the mixtures are dominated by a very small fraction of the compounds present. The values of HI were correlated with *n*, indicating that samples with more detected compounds in general had higher estimates of cumulative toxicity. In contrast, MCR values had little or no increase with *n*. This suggests that in this dataset, higher numbers of chemicals in a mixture do not necessarily indicate an increased need for a cumulative risk assessment. By plotting MCR *versus* HI the analysis demonstrated that many samples have a toxicity that may be seven-fold greater than the toxicity of any one chemical component. However, for the mixtures with higher toxicity (HI values greater than 1) the difference was less than three-fold for all of the samples and averaged only 1.3 fold. The finding of a negative correlation of MCR with HI suggests that the toxicities of the mixtures of the greatest concern are driven by smaller numbers of compounds than the mixtures with minimal toxicity. This implies that the higher toxicity in these samples did not occur as a result of the contribution of multiple chemicals summing to unacceptable levels of toxicity, but rather from the presence of one (or two) chemicals that either were highly toxic or occurred at high concentrations. The final decision on the need to perform a cumulative risk assessment will be determined by many factors; however, in this case performing a Tier 1 cumulative risk assessment would result in only modest changes in the predictions of risk for the more toxic mixtures.

This assessment has focused on cumulative exposures to PPPs and degradation products of PPPs that result from the co-occurrence of the chemicals in surface water samples. The approach can be applied to cumulative exposures that occur from exposures to mixtures of chemicals in soil, air, or on indoor surfaces. The approach can also be applied to cumulative exposures to chemicals from multiple sources when the doses of the chemicals can be defined for a single individual. While not discussed, the MCR approach could be extended to consider non chemical stressors when the impact of those stressors on the toxicity of the chemicals is defined. Finally, as discussed above, the purpose of this analysis is the illustration of the application of the MCR to a real world dataset. The purpose is not to reach any conclusion on the safety of current levels of PPPs in U.S. surface waters. The levels observed in the NAQWA dataset are measures of conditions one to two decades ago and do not necessarily reflect current practices in the U.S. In addition, the exposure assumptions used in this analysis will lead to significant overestimates of actual chronic exposures the mixtures of chemicals in the samples. Many of the samples are taken from surface waters that are not appropriate for drinking water supplies (small streams) and the impacts of water treatment processes on the levels of PPPs are not considered. Under a WHO tier 1 assessment, value of HI greater than 1 indicate that the samples would pass on to Tier 2 and Tier 3 assessments where more realistic exposure and toxicity assumptions would be used [7].

Conclusions

MCR may provide a useful tool to assess the value of performing a cumulative risk assessment. The approach can be extended to cumulative exposures involving large numbers of chemicals and can be applied to large monitoring datasets. The approach can be applied as part of Tier 1 assessments that use simple additive models of toxicity. The findings provide a quantitative estimate of individuals' toxicities missed by not performing a cumulative risk assessment. As a result, MCR values could be used as part of a decision process to determine when, and where, future cumulative risk assessments are most needed to protect human health.

Acknowledgements

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Potential Conflicts of Interest

The Dow Chemical Company manufactures many chemicals substances including some of the PPPs listed in Table 1. However, since this manuscript only addresses methodological issues the authors declare no conflict of interest.

References

1. National Research Council. *Science and Decisions: Advancing Risk Assessment*; Committee on Improving Risk Analysis Approaches Used by the U.S. EPA, National Academies Press: Washington, DC, USA, 2009.
2. National Research Council. *Phthalates and Cumulative Risk Assessment*; National Academy Press: Washington, DC, USA, 2009.
3. U. S. EPA. *Concepts, Methods and Data Sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures and Effects: A Resource Document*; U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment: Cincinnati, OH, USA, 2007.
4. The School of Pharmacy, University of London; Göteborg University; Faust & Backhaus; Environmental Consulting GbR. *State of the Art Report on Mixture Toxicity*; Directorate-General for the Environment (DG Environment), European Commission: Brussels, Belgium, 2009.
5. *Chemical Mixtures: A Framework for Assessing Risks to Human Health*; Interdepartmental Group on Health Risks from Chemicals (IGHRC), Institute of Environment and Health: Cranfield, UK, 2008.
6. World Health Organization. *Harmonization Project Document 7; Assessment of Combined Exposures to Multiple Chemicals: Report of a WHO/IPCS International Workshop on Aggregate/Cumulative Risk Assessment*; WHO: Geneva, Switzerland, 2009.
7. Meek, M.E.; Boobis, A.R.; Crofton, K.M.; Heinemeyer, G.; Van Raaij M.; Vickers, C. Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. *Regul. Toxicol. Pharmacol.* **2011**, *60*, S1-S14.
8. Koneman, H. Fish Toxicity Tests with Mixtures of More than Two Chemicals: A Proposal for a Quantitative Approach and Experimental Results. *Toxicology* **1981**, *19*, 229-238.
9. Junghans, M.; Backhaus, T.; Faust, M.; Scholze, T.; Grimme, LH. Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. *Aquat. Toxicol.* **2006**, *76*, 93-110.
10. Gilliom, R.J.; Barbash, J.E.; Crawford, C.G.; Hamilton, P.A.; Martin, J.D.; Nakagaki, N. *The Quality of Our Nation's Waters—Pesticides in the Nation's Streams and Ground Water*, *1992–2001*; U.S. Geological Survey: Reston, VA, USA, 15 March 2006.
11. Helsel, D. Much ado about next to nothing: incorporating non-detects in science. *Ann. Occup. Hyg.* **2010**, *54*, 257-262.
12. *Pesticides in the Nation's Streams and Ground Water, 1992–2001*. Available online: http://water.usgs.gov/nawqa/pnsp/pubs/circ1291/appendix6/ (accessed on 22 November 2010).

Chapter 8

Using Information on Exposure to Characterize Risks to Human Health from Concurrent Exposures to Multiple Chemicals: Summary

This thesis has explored the concept of using exposure information to understand, organize, and manage the risks associated with cumulative exposures. The work presented in this thesis demonstrates how a careful consideration of exposure information can:

- Assist in the problem formulation step in cumulative risk assessment;
- Show how screening assumptions can be used for estimating toxicity of certain chemicals in mixtures;
- Identify which chemicals in an individual's cumulative exposure have the greatest potential to interact and produce a super-additive response; and
- Measure how much toxicity is "missed" by not performing a cumulative risk assessment.

It is critical, however, to have access to the exposure information necessary to support a cumulative risk assessment. As a result, the thesis begins with the development of a tool to characterize the exposure data needed to perform a cumulative exposure assessment.

Focus 1. Use of simulation models to characterize cumulative exposures to chemicals.

Historically, the focus on chemical-specific assessments has resulted in research programs that report data on exposure to specific chemicals. This can, for example, take the form of summary tables where select percentiles or moments of the cumulative distributions of exposure levels or media concentrations across a survey population are reported. While such tables provide a convenient way of summarizing data and can be helpful in comparing chemicals' relative exposures, they are not sufficient for cumulative risk assessments. What is necessary for such assessments are data on which chemicals reach a specific individual and in what amounts. In the case of monitoring this means access to the raw data from the monitoring surveys rather than summary tables. In the case of data from exposure models, this will require predictions of the doses to a specific individual with consistent characteristics.

Chapter 3, presented a modelling framework, person oriented modelling (POM), for addressing interindividual variation in exposures, longitudinal variation in individual's exposures, exposures from multiple sources, and multiple chemicals, and finally the uncertainty in the estimates. Building models around the person provides a number of advantages over traditional modelling approaches.

First, data on exposures to statistically representative individuals are essential for cumulative risk assessments since it is the characterization of risks to persons and how they change as a function of mitigation that is the ultimate purpose of the model. Second, inter- and intra- individual variation in personal doses and risks for a population and at a specific time are key outputs for the model. Third, defining internally consistent doses from exposures to multiple sources and routes of exposure to one chemical, or to multiple chemicals, can only occur if the same person is modelled for each source. This cannot be achieved by simply summing the outputs of separate source to outcome models. Finally, focusing the modelling on the person allows for the calculation of doses from the person's exposures that are consistent with the time of exposure, location, and age of the individual.

POM has the additional benefit of efficient programming. The complexity of a program designed using POM increases linearly with the number of sources. This is in contrast to programs such as decision tree analysis where model complexity and run times increases geometrically. This means that large numbers of sources can be included in one POM model [1]. In 2011, software based on this approach was used as the basis for modelling aggregate dietary exposures to chlorpyrifos and to model the impact of the pesticide on individual's cholinergic system [2-4]. The resulting model defined the individual and used the definition to estimate physiology, diet, metabolism for the individual. This model design has the potential to be extended to include the cumulative effects from multiple pesticides that target cholinesterases [4].

POM raises a number of implications that go beyond the design of software. First it suggests that the problem formulation for cumulative exposures needs to move from a linear approach (Figure 1) to a person-centric model (Figure 2).

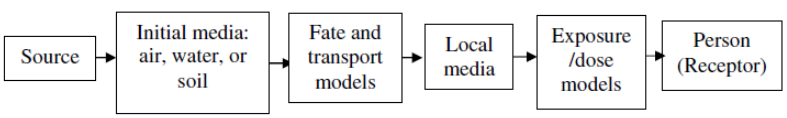

Figure 1. Traditional source to person model.

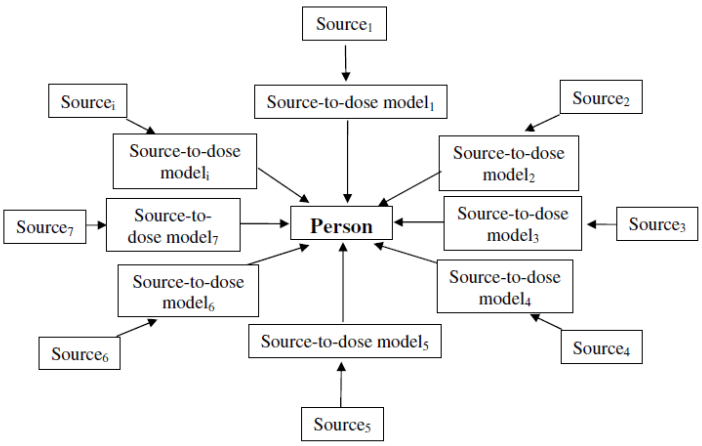

Figure 2. Person oriented model.

POM based models leads to the concept that the community rather than the individual sources should be the focus of the exposure and risk assessment process. The communities, collections of individuals who receive the doses from the sources, become the frameworks for performing cumulative exposure and risk assessments [5]. This suggests that cumulative exposure assessments performed on one community may be difficult to extrapolate to other communities. When the communities are very different (urban neighbourhood versus rural towns) separate assessments may be required.

Chapter 2 of the thesis presents an example application of the POM modelling approach. The example presented an analysis of cumulative exposures to three pesticides. The pesticides were used either on crops or in individuals' residences. The agricultural uses resulted in residues in foods and in public water supplies from ground and surface water contamination. Residential sprays to control insects resulted in exposures by the dermal, oral, and inhalation pathways. As a result the model simulated the aggregate exposures to each pesticide and the cumulative exposure to all three pesticides. The risk was assessed using an additive model of exposure. The assessment used hypothetical data, but the data were designed to be a realistic description of pesticides used at the time of the analysis and that could be provided by industry as part of a registration process.

The key finding from the effort was that the model produced the data needed by cumulative risk assessments. In this case the data included the multi-route, multi-source, and multi-chemical doses for each simulated individual at each point in

time. The model characterized both inter-individual variations in exposure and the variation in individuals' exposures over time.

Focus 2. Developing a framework to assessing the risk management implications of toxicological findings of synergy.

Currently, synergy is an issue that while acknowledged is not quantitatively considered in most frameworks proposed for cumulative exposure [6-9]. Chapter 4 reviews the concept of synergy and its potential for the impact on regulation of cumulative exposures or exposures to mixtures. The chapter focused on exposures to mixtures of chemicals. However the approach developed can be applied to individuals' cumulative exposures to chemicals that occur from multiple sources. This chapter outlined a series of arguments on the potential for the occurrence of synergy in humans exposed to the doses permitted under additive or independence models of mixture toxicity commonly used by risk assessors.

The chapter argued that synergy is only been reported to occur when humans or test animals receive high doses of chemicals that that are sufficient to independently cause adverse effects [10, 11] (i.e., frank effect levels). In contrast, the use of either additive or independence models to determine safe levels of cumulative exposures limits chronic exposures to chemicals to levels that are below their regulatory criteria (such are Reference Doses, Allowable Daily intakes, or Derived No Adverse Effect Levels). In simple binary mixtures, the permitted exposures could in theory approach the component chemical's regulatory criteria; however, if the mixtures include larger numbers of components, the mixtures' components will be restricted to much lower doses.

The chapter examined a real world mixture of five organophosphorous pesticides [12, 13]. When an additive model was used to determine a safe dose of the mixture it was found that the largest exposure permitted for each of the individual pesticides ranged from one half to less than one seventieth of the pesticides regulatory standards. In addition, current conservative practices in setting regulatory standards (when extrapolating from animal data to sensitive humans) have the net effect of further reducing the ratio of the permitted doses of the mixture components to actual population thresholds of the chemicals.

As a result of the differences in doses, the key regulatory question is "do findings of synergy at frank effect levels imply that synergy would occur at the doses permitted under additive models?" The consensus from the toxicological was that synergy was dose dependent and thus could not be assumed at all doses of a

mixture. Specifically, synergistic effects of mixtures have been shown to have thresholds (thresholds of interaction) below which the mixtures follow additive or independence models [14].

The chapter also presented a methodology that can be used to evaluate the regulatory significance of specific findings of synergy and synergy thresholds. Using this methodology, risk assessors can determine if synergy is likely to occur in a specific set of doses that reach an individual and determine the specific chemicals where synergistic effects are most likely to occur. In the future this method could be used to evaluate all claims of synergy to determine if the findings indicate that additive models may not be protective.

Focus 3. Screening approaches for assessing the toxicity of mixture components with no data.

Chapters 5 and 6 demonstrate how the Cramer class portion of the Threshold for Toxicological Concern can be used to fill data gaps in mixture risk assessment by providing conservative estimates of compounds with missing toxicity data. Chapter 5 presented the methodology and applied it to a set of mixture of anthropogenic chemicals reported to occur in samples of surface waters. Chapter 6 presents an application of the approach to trace level of chemicals extracted from food contact materials.

As discussed in Chapter 1 component-based models of mixture toxicity require toxicity data on every component of a mixture. Thus in theory missing the data on a single chemical would make a cumulative assessment of risk impossible. The approach presented in chapters 5 and 6 addresses this problem by developing conservative estimates of chronic toxicity using the structure of the compounds. This approach is an example of a Tier 0 assessment under the WHO approach for assessing risks from mixtures (see Annex B of Meek et al. [15]). The approach can support the development of component-based estimates of mixture toxicity for mixtures where there are no toxicity data on any mixture components. In these situations the only data that are required are the weight fraction of each component in the mixture and the chemical structures of the components. In addition, Cramer classes can be used to fill in missing data for mixtures where data exist on a portion of a mixture's components.

The values of toxicity produced by use of Cramer classes, however, have a significant potential to overestimate the toxicity of mixtures. As demonstrated in Chapter 5, when the toxicity of the all the components of mixtures were defined

using the Cramer classes, the toxicity of the mixtures were overestimated by up to three orders of magnitude. The degree of overestimation was minimized if the dominant compound (having the largest hazard quotient) in the mixture was based on actual data. This suggests that when a Cramer class is used to provide toxicity estimates for one or more components, the user should determine if any of these components become the dominant component of the mixture. If this is the case then the prediction of the toxicity of the mixture should be taken with caution. This limitation does not mean that such estimates are without value. Such conservative estimates may find considerable use in screening out low-risk mixtures.

This study also investigated the use of probabilistic models of the uncertainty in the estimates of mixture toxicity. The analyses confirm that the deterministic predictions are conservative. The concern that the application of conservative assumptions to multiple chemicals would overestimate the toxicity of mixtures did not prove to be an important issue since most mixtures had their toxicities driven by only one or two chemicals. However, the uncertainty analysis still provides significant benefits. The use of probabilistic techniques improves the risk assessment process by providing the risk manager with a context for single value measures of toxicity. For example, providing the median value of the uncertainty distribution in the mixture's Derived No Effect Levels discloses the degree of precaution built into the deterministic estimates. In addition, the use of the 5th percentile of the uncertainty distributions provides a more objective and consistent measure of toxicity across different mixtures. This consistency could improve comparative risk determinations.

In Chapter 6 the Crammer class approach was used to assess risks from mixtures of chemicals that leach from food contact materials (called migrating chemicals or "migrants"). Existing approaches for assessing the risks from these migrating chemicals have assessed each chemical separately. Combination of existing mixture models, the use of Cramer classes to estimate toxicity, and probabilistic modelling of the uncertainty in estimates of safe levels of exposure can provide useful characterizations of the toxicity of the mixtures of migrants. The proposed tool has the ability to:

- Estimate the toxicity of mixtures composed of any number of migrants;
- Estimate the toxicity of mixtures that include certain organic chemicals with no toxicity data;
- Allow the use of toxicity information on individual chemicals (when it is available) to improve the assessment of mixture toxicity; and
- Quantify the uncertainty in the predicted safe doses.

However, the approach produces estimates that are biased towards overestimating toxicity. As a result, the approach will be most useful in screening out low-risk mixtures.

Focus 4. Use of exposure information to evaluate the need for performing cumulative risk assessments.

Chapter 7 presents the concept of the MCR. This tool has been shown to be a simple but powerful method for evaluating the value of performing a cumulative exposure of a population. Cumulative exposure and risk assessments require significant levels of resources and when chemical-by-chemical approaches are sufficient to identify the subpopulations that are at risk then society benefits from avoiding unnecessary assessments. Values of MCR that are close to 1 indicate that a cumulative assessment would have little value over a chemical-by-chemical approach. MCR values of 2 indicate that half of the risk would be missed. MCR values greater than 2 indicate that the majority of the risk would be missed.
MCR values plotted against mixtures' toxicities provide a useful way to identify specific mixtures that are most in need of cumulative assessments.

This chapter describes the MCR and an application of the methodology to humans' hypothetical exposures to mixtures of pesticides in surface water. The data on the pesticides came from a large U.S. survey 3,099 samples performed from 1991 to 2000. MCR values were determined for the cumulative toxicities of the chemical mixtures in the surface water samples. The number of chemicals detected in the samples ranged from 5 to 29. Values of MCR range from 1.0–4.0 (mean of 1.8). This indicates that the toxicities of all of the mixtures are dominated by a very small fraction of the compounds present. The values of MCR values had little or no increase with the number of compound in a mixture. This suggests that in this dataset, higher numbers of chemicals in a mixture do not necessarily indicate an increased need for a cumulative risk assessment. By plotting MCR *versus* HI the analysis demonstrated that many samples have a toxicity that may be four-fold greater than the toxicity of any one chemical component. However, for the mixtures with higher toxicity (HI values greater than 1) the difference was less than three-fold for all of the samples and averaged only 1.3 fold. This suggested that a chemical-by-chemical approach would have identified the majority of the mixtures of concern.

PERATO PRINCIPLE AND CUMULATIVE EXPOSURES

Throughout this thesis evidence was found that the risk from cumulative exposures to combinations of chemicals follow the Perato Principle [16]. The Perato principle states that the majority of any factor that is derived from multiple sources comes from a minority of the sources. In the case of cumulative risks from chemicals the specific finding is that the majority of the risk comes from a minority of chemicals.

In Chapter 2 when the sources of pesticide are independent, aggregate exposures for most individuals in the general population tend to be dominated by a single route of exposure. In addition, when an additive model of cumulative toxicity is used, most individuals have their cumulative doses dominated by one of the three pesticides. This can be seen in the following figure taken from Chapter 2 that presents the cumulative doses of the three pesticides that had been normalized to an index chemical (in this case pesticide Alpha) and the sum of the three doses across the simulated population. For individuals in the community with typical exposures, pesticide "Beta" dominates exposures. But for the fraction of the community with highest exposures (top 2%) the individuals' exposures are dominated by pesticide Gamma.

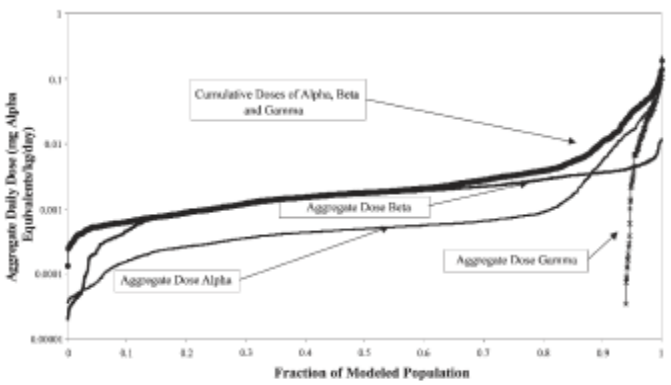

Fig. 5. This figure presents the maximum seasonal aggregate doses of Alpha, Beta, Gamma and cumulative dose of all three pesticides during winter for individuals aged three. All four dose distributions have been ranked separately. Units of dose are in mg of the index pesticide (Alpha).

In Chapter 4 the results from a study of five pesticides where the doses were based on the pattern of doses observed in dietary studies. In this mixture one pesticide was found to dominate the toxicity of the mixture.

Chapter 5 found that additive and independence models produce similar results in the 48 surface water samples differing by less than a factor of 3 and on average differed by a factor of 1.4. Junghans et al. [17] demonstrated that the predictions of additive and independence models would agree only when a single compound dominated the toxicity of the mixture.

In Chapter 7, the MCR values for exposures to mixtures of pesticides were found to range from 1-4 and have an average value of 1.8. Values of MCR less than 2 indicate that a single chemical is providing more than 50% of the toxicity of the mixture. This was observed in mixtures that included up to 29 pesticides.

While proof that all mixture exposures and all cumulative exposures will follow the Perato principle is beyond the scope of this thesis, the finds suggest that this is a topic that should be investigated in the future.

Chapter 7 also presented a finding that is related to the Perato Principle, but goes beyond this concept. The finding was that the MCR values of mixtures were inversely related to the toxicities of the mixtures as measured by the hazard index (HI). Additional evidence for an inverse relationship between MCR and toxicity has been observed in drinking water exposures to ground water contaminants [18] and in biomonitoring studies of dioxin-like compounds [19].

This finding has a number of interesting implications. First, the finding of a negative correlation of MCR with HI suggests that the toxicities of the mixtures of the greatest concern are driven by smaller numbers of compounds than the mixtures with minimal toxicity. This implies that the higher toxicity in these samples did not occur as a result of the contribution of multiple chemicals summing to unacceptable levels of toxicity, but rather from the presence of one (or two) chemicals that either were highly toxic or occurred at high concentrations. Second, the negative correlation suggests that the current system of chemical-by-chemical assessment may be unusually efficient in identifying cumulative exposures of concern. This occurs because the difference between chemical-by-chemical and cumulative exposure assessment are smaller for more toxic mixtures. It also suggests that finding of differences between chemical-by-chemical and cumulative exposures for mixture of low toxicological concern do not automatically prove that similar differences occur for mixtures with higher toxicities. Third, it suggests that the longstanding debate of whether additive or independence model are most appropriate for evaluating mixtures may have less value then was thought in the past.

Again, demonstration of whether this phenomenon occurs for all mixtures and all cumulative assessments goes beyond the scope of this thesis. The MCR approach will need to be applied to other cumulative exposures for other sources (diet, indoor air, consumer products, etc.) to determine if these cumulative exposures follow a similar pattern. Clearly this is a finding that needs additional investigation.

References

1. Price P, Chaisson C, Koontz M, Ryan B, Wilkes C, Macintosh D, Georgopoulos P. 2003. Construction of a comprehensive chemical exposure framework using person-oriented modelling. The Lifeline Group. Developed for The Exposure Technical Implementation Panel American Chemistry Council Contract. # 1388. Available online at http://www.thelifelinegroup.org/lifeline/documents/
2. comprehensive_chemical_exposure_framework.pdf
3. Hinderliter PM, Price PS, Bartels MJ, Timchalk C, Poet TS. Development of a source-to-outcome model for dietary exposures to insecticide residues: An example using chlorpyrifos. Regul Toxicol Pharmacol. 2011 Volume 61, Issue 1, October 2011, Pages 82-92
4. Price PS, Schnelle KD, Cleveland CB, Bartels MJ, Hinderliter PM, Timchalk C, Poet TS. Application of a source-to-outcome model for the assessment of health impacts from dietary exposures to insecticide residues. Regul Toxicol Pharmacol. 2011 Volume 61, Issue 1, Pages 23-31.
5. DAS. 2010. Source-to-Outcome Modelling Physiologically Based Pharmacokinetic/ Pharmacodynamic (PBPK/PD) Model Linked to a Dietary Exposure Model: Chlorpyrifos as a Case Study, Science Issues Paper for SAP Meeting on Chlorpyrifos Physiologically Based Pharmacokinetic/Pharmacodynamic (PBPK/PD) Model linked to the Cumulative and Aggregate Risk Evaluation System (CARES) , Dow AgroSciences, February 15-18, 2011, Washington, D.C.
6. Callahan MA and Sexton K, 2007. If Cumulative Risk Assessment Is the Answer, What Is the Question?, Environ Health Perspect. 2007 May; 115(5): 799–806
7. Scientific Committees (Scientific Committee on Consumer Safety (SCCS) Scientific Committee on Health and Environmental Risks (SCHER) Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). 2011. Toxicity and Assessment of Chemical Mixtures, European Commission, DG Health & Consumers, December, 2011
8. Cefic. 2011. Comments provided to on the Scientific Committees report Toxicity and Assessment of Chemical Mixtures, 16 September
9. WHO. 2009. Harmonization Project Document 7; Assessment of Combined Exposures to Multiple Chemicals: Report of a WHO/IPCS International Workshop on Aggregate/Cumulative Risk Assessment; WHO: Geneva, Switzerland
10. Kortenkamp A, Backhaus T, Faust M. 2009. State of the Art Report on Mixture Toxicity Final Report, Study Contract Number 070307/2007/485103/ETU/D.1, 22 December
11. 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, Zaleski R. 2011. Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment. Crit Rev Toxicol. 2011 Feb 10.
12. ECETOC, Workshop on Combined Exposure to Chemicals. 11-12 July 2011, Berlin
13. Gennings, C., Carter, J.W.H., Casey, M., Moser, V., Carchman, R., and Simmons, J.E. (2004) Analysis of functional effects of a mixture of five pesticides using a ray design. Environ. Tox. Pharmacol., 18, 115–125.
14. Moser, V.C., Simmons, J.E., and Gennings, C. (2006) Neurotoxicological interactions of a five-pesticide mixture in preweanling rats. Toxicol. Sci., 92, 235–245.

15. Yang R, Dennison J. 2007. Initial analyses of the relationship between "Thresholds" of toxicity for individual chemicals and "Interaction Thresholds" for chemical mixtures. Toxicol Appl Pharmacol. 223(2):133-8.
16. Meek M, Boobis A, Crofton K, Heinemeyer G, Van Raaij M, Vickers C. 2011. Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. Regul. Toxicol. Pharmacol. Vol. 60, (S1-S14)
17. Juran JM. 1950. Pareto, Lorenz, Cournot, Bernoulli, Juran and others. Industrial Quality Control (October): 25.
18. Junghans, M.; Backhaus, T.; Faust, M.; Scholze, T.; Grimme, LH. Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. Aquat. Toxicol. 2006, 76, 93-110.
19. Han X, Price P. 2011. Determining the Maximum Cumulative Ratios for Mixtures Observed in Ground Water Wells Used as Drinking Water Supplies in the United State Int. J. Environ. Res. Public Health 2011, 8(12), 4729-4745; doi:10.3390/ijerph8124729.
20. Han X, Price P. 2011. Determining the Maximum Cumulative Ratios for Dioxin-like Compounds in the General Public and Occupationally Exposed Individuals Using Biomonitoring Data, The Journal of Exposure Science and Environmental Epidemiology, In press 2012

Chapter 9

Summary and recommendations

The practice of cumulative risk assessment is astounding for its lack of progress. As discussed in Chapter 1 the basic concepts of additivity and independence were outlined in 1939 [1]. The current model of additivity was first proposed as a risk management tool in 1963 [2]. The issue of mixtures in the environment was identified in 1980 [3]. In 2012 the issue of the magnitude of the risks posed by cumulative exposures to chemicals remains largely unresolved with only a small portion of cumulative exposures from a limited number of sources are evaluated on a systematic basis [4]. One of the reasons for this lack of progress is the sheer size of the issue of mixtures. While the current TSCA inventory lists 82,000 chemicals potentially in commerce [5], this number is dwarfed by the number of mixture and combinations of doses that make up current cumulative exposures. Clearly what is required are tools to more efficiently determine cumulative exposures and the risks they pose, but more importantly tools for identifying when and where cumulative risks will pose unacceptable risks.

The concepts and approaches outlined in this thesis are an attempt to move the issue of assessing cumulative effects of chemical exposures forward. Chapters 2 and 3 present a modelling framework for addressing interindividual variation in cumulative exposures. Chapter 4 investigates the issue of synergy and presents arguments that support the current use of additive models as conservative screens for assessing the risk of effects from cumulative exposures. Chapters 5 and 6 demonstrate how the Cramer class portion of the Threshold for Toxicological Concern can be used to fill data gaps in mixture risk assessment by providing conservative estimates of compounds with missing toxicity data. Chapter 7 presents the concept of the Maximum Cumulative Ratio. This tool has been shown to be a simple but powerful tool in demonstrating the importance of performing a cumulative exposure.

Two ideas have emerged from the body of work. First, it is critical to determine if the thresholds of interaction for chemicals in a cumulative assessment are always higher than the lowest threshold for an individual chemical. If this is true then the current approach of using additive models as a screen will be protective because additive models will keep all doses below the threshold of interaction. Future work is needed to assess if this is always the case. It is possible that the rise of High Throughput Assays using human cells and tissues can provide insights on this issue.

Second does the finding that MCR values in individuals' cumulative exposures decrease as the cumulative toxicity increases consistently occur for all cumulative exposures. Future work will need to determine if cumulative exposures to other sources of exposure (e.g., dietary, indoor air, or dermal contact to consumer products) confirm if the patterns of smaller values of MCR with exposures to mort

toxic chemicals observed in existing studies of drinking water exposures are a universal phenomena or if there are exceptions.

Recommendations

It is hope of the author that the tools for assessing cumulative exposures, evaluating findings of synergy, filling data gaps in toxicity information, and evaluating the need for cumulative risk assessments will be used by researchers in the future. Too often in the past, chemical regulations have tended to shoot first and aim second. Using the MCR and the other approaches discussed in this thesis, researchers can identify those sources and chemicals that drive cumulative risks and those individuals most in need of protection. Such information can help guide both industry's and government's management of risks from cumulative exposures.

This work has already begun with the application of the MCR approach to the human health effects from exposures to other to natural and anthropogenic chemicals in groundwater wells [6] and the patterns of exposure to dioxin like exposures [7]. Currently, a project is underway to apply the MCR to the human health and environmental effects associated to exposures to mixtures in surface waters [8]. A second project will investigate concurrent exposures to chemicals in indoor air has also recently been initiated.

References

1. Bliss C. 1939. The Toxicity of Poisons Applied Jointly. Annals of Applied Biology. 26:585-615
2. ACGIH. 1963. Transactions of the Twenty-Fifth Annual Meeting of the American Conference of Governmental Industrial Hygienists, May 6–10, Cincinnati, Ohio.
3. National Research Council. 1980. Problems of Risk Estimation Drinking Water and Health, Volume 3. Washington, DC: The National Academies Press
4. Kortenkamp A, Backhaus T, Faust M. 2009. State of the Art Report on Mixture Toxicity Final Report, Study Contract Number 070307/2007/485103/ETU/D.1, 22 December
5. *TSCA inventory accessed 16 January 2012*
6. *http://www.epa.gov/oppt/existingchemicals/pubs/tscainventory/basic.html*

7. Han X, Price P. 2011. Determining the Maximum Cumulative Ratios for Mixtures Observed in Ground Water Wells Used as Drinking Water Supplies in the United State Int. J. Environ. Res. Public Health 2011, 8(12), 4729-4745; doi:10.3390/ijerph8124729.
8. Han X, Price P. 2012. Determining the Maximum Cumulative Ratios for Dioxin-like Compounds in the General Public and Occupationally Exposed Individuals Using Biomonitoring Data Submitted to the Journal of Exposure Science and Environmental Epidemiology, In Press April 2012
9. Price P, Dhein E, Hammer M, Heneweer M, Junghans M, Kunz P, Magyar C, Penning H, Rodriguez C, 2012 A decision tree for assessing effects from exposures to multiple substances Submitted May 2012 Environmental Sciences Europe

Chapter 10

Nederlandse Samenvatting
(Dutch Summary)

In dit proefschrift wordt nader ingegaan op het gebruik van blootstellingsinformatie om de risico's verbonden aan cumulatieve blootstelling (blootstelling aan verschillende chemicaliën van diverse bronnen) te begrijpen, reguleren en beheren. Over het algemeen is het verbazingwekkend hoe weinig vooruitgang er tot nu toe is geboekt in de toepassing van de cumulatieve risicobeoordeling. Cumulatieve risicobeoordeling is al meer dan dertig jaar geleden op de agenda gezet. Nu, anno 2012, blijkt slechts een klein deel van de cumulatieve blootstelling die afkomstig is van een beperkt aantal bronnen op systematische wijze geëvalueerd te worden. Eén van de redenen voor dit gebrek aan vooruitgang is de enorme omvang van de mengseltoxiciteitsproblematiek. In de Verenigde Staten vermeldt het Toxic Substances Control Act (TCA) overzicht circa 82000 chemicaliën, die commercieel gebruikt worden. Dit aantal valt in het niet in vergelijking tot het aantal mengsels en combinaties van doseringen die kunnen voorkomen. Dit voorbeeld maakt duidelijk dat er nieuwe methoden nodig zijn om op efficiëntere wijze de risico's van cumulatieve blootstellingen te bepalen. Nog belangrijker zijn echter (nieuwe) methoden voor het bepalen van waar en op welk moment een cumulatieve blootstelling aan stoffen leidt tot onaanvaardbare risico's voor mens en milieu.

Zoals beschreven en bediscussieerd in hoofdstuk 1 zijn de fundamentele concepten van "additivity" en "independent action" reeds gedefinieerd in 1939. Het huidige additiviteitsmodel werd reeds in 1963 voor de eerste keer voorgesteld als een methode voor risicomanagement. De mengseltoxiciteitproblematiek in het milieu kreeg pas in 1980 de noodzakelijke aandacht. Momenteel is de omvang van de risico's van cumulatieve blootstellingen aan chemicaliën voor een groot deel nog steeds onbekend. In de dagelijkse praktijk wordt nog steeds maar een klein deel van de cumulatieve blootstellingen die afkomstig zijn van een beperkt aantal bronnen op systematische wijze geëvalueerd.

De concepten en benaderingen, zoals beschreven in dit proefschrift, zijn een poging om de beoordeling van cumulatieve effecten van chemische blootstellingen meer onder de aandacht te brengen. De hoofdstukken 2 en 3 beschrijven een model om de interindividuele variatie in cumulatieve blootstelling nader te bepalen. Hoofdstuk 4 gaat nader in op het aspect van mogelijke synergistische effecten bij mengselblootstelling. Naar aanleiding hiervan worden argumenten aangedragen die het huidige gebruik van additiviteitsmodellen als een conservatieve methode voor de risicobepaling van cumulatieve blootstelling aan stoffen nader ondersteunen. Hoofdstuk 5 en 6 tonen aan hoe de zogenaamde "Cramer class portion of the Threshold for Toxicological Concern" gebruikt kan worden om de ontbrekende gegevens in de risicobeoordeling van chemicaliën aan te vullen. Hierbij kan gebruik gemaakt worden van conservatieve schattingen van de risico's van stoffen waarvoor feitelijke toxiciteitgegevens ontbreken. Hoofdstuk

7 beschrijft het concept van de maximale cumulatieve verhouding (Maximum Cumulative Ratio (MCR)). Dit blijkt een eenvoudige en krachtige methode te zijn, die het belang aantoont voor het uitvoeren van een cumulatieve blootstelling in de risicoschatting.

Op basis van het onderzoek beschreven in dit proefschrift kunnen er twee belangrijke aanbevelingen gegeven worden voor de risicobeoordeling van mengsels en cumulatieve blootstelling. Ten eerste is het belangrijk om te bepalen of de drempelwaarde voor interactie van de chemicaliën in een cumulatieve blootstelling altijd hoger is dan de laagste drempelwaarde voor een individuele stof. Als dit zo is, dan is de huidige benadering met het gebruik
van additieve modellen beschermend genoeg. In dit geval zijn doses beneden de interactiedrempel. Toekomstig onderzoek zal moeten aantonen of deze situatie altijd van toepassing is. Wanneer in de toekomst vaker gebruik gemaakt gaat worden van zogenaamde “High Throughput Assays” met humane cellijnen en weefsels, kan op dit gebied meer inzicht worden verkregen.

Ten tweede is het van belang te weten of de MCR waarden in de individuele cumulatieve blootstelling afnemen, wanneer de cumulatieve toxiciteit consistent toeneemt voor alle cumulatieve blootstellingen. Toekomstig onderzoek moet aantonen of cumulatieve blootstellingen aan andere blootstellingsbronnen (bijvoorbeeld via de voedselketen, binnenlucht of huidcontact met consumentenproducten) bevestigen of de patronen van lagere MCR waarden voor blootstelling aan mengsels van toxische stoffen, zoals onder andere waargenomen bij blootstelling via drinkwater, een normaal verschijnsel is of eerder een uitzondering.

De auteur hoopt dat de in dit proefschrift beschreven methoden voor: het bepalen van cumulatieve blootstellingen, het evalueren van synergistische effecten en het opvullen van kennishiaten in toxicologische informatie in de toekomst toegepast zullen gaan worden. In deze context moet geconstateerd worden dat in het verleden bij de chemische regelgeving te veel de neiging bestond om te handelen zonder een duidelijk doel voor ogen te hebben. Door gebruik te maken van de MCR methode en andere benaderingen die beschreven zijn in dit proefschrift, kunnen onderzoekers zowel de bronnen en chemicaliën die de cumulatieve risico’s bepalen, maar ook de personen die hiervoor de meeste bescherming nodig hebben identificeren. Dergelijke informatie kan zowel het risicomanagement bij de industrie als de overheid helpen in het beheersen van de risico’s van cumulatieve blootstelling.

Deze benadering is reeds begonnen met de toepassing van de MCR benadering voor het bepalen van humane gezondheidseffecten door blootstelling aan

combinaties van voorkomende en antropogene chemicaliën in grondwaterbronnen of humane blootstellingpatronen aan dioxineachtige verbindingen. Momenteel loopt er een onderzoeksproject waarin de MCR voor de humane gezondheids- en milieu-effecten gekoppeld wordt aan de blootstelling aan mengsels van stoffen in oppervlakte wateren. Een ander project, waarin de gelijktijdige blootstelling aan mengsels van chemicaliën in het binnenmilieu zal worden onderzocht, is recentelijk opgestart.

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CURRICULUM VITAE

Paul S. Price was born in 1952 and has a B.A. in Chemistry (1975) and M.S. in Civil Engineering (1979) from the University of Maryland. He also holds a graduate degree in theology from Regent College, Vancouver, Canada (1976). Mr. Price joined the U.S. Environmental Protection Agency in 1979 as an early practitioner in the field of exposure and risk assessment. He left EPA in 1987 to join the American Petroleum Institute to address issues related to the regulation of air emissions, hazardous waste, and exposures to benzene. In 1991 he became a consultant on risk and exposure issues for government and industry. In 1999 he cofounded The LifeLine Group, a nonprofit organization that builds software that assesses dietary and residential exposures. In 2006 he joined The Dow Chemical Company as a Risk Assessment Leader. At Dow he has directed a research program to assess risks from mixtures. He is currently leading Dow's program on cheminformatics. He has published more than 50 papers and book chapters. In his spare time, he studies the impact of science and technology on society as reflected in the popular cultures of the United States and Japan.

PUBLICATIONS

1. Saghir SA, McFadden LG, Bartels MJ, Rick DL, Price PS, Fontaine DD. 2012 Statistical methodology to determine kinetically derived maximum tolerated dose in repeat dose toxicity studies Reg Tox Pharm In press
2. McNally K, Cotton R, Cocker J, Jones K, Bartels MJ, Rick D, Price PS and Loizou GD. 2012, Reconstruction of Exposure to m-Xylene From Human Biomonitoring Data Using PBPK Modelling, Bayesian Inference, and Markov Chain Monte Carlo Simulation, Journal of Toxicology In press
3. Pottenger LH, Price PS, Gollapudi BB, 2012, Needed: Improved Dose Selection in Genetic Toxicology Studies, Human and Ecological Risk Assessment: An International Journal, Available online: 13 Jan 2012
4. CV Rider; M Dourson; RC Hertzberg; MM Mumtaz; PS Price; JE Simmons, 2012. *Incorporating Nonchemical Stressors Into Cumulative Risk Assessments,* Toxicological Sciences 2012; doi: 10.1093/toxsci/kfs088
5. Price P and Han X. 2011. Maximum Cumulative Ratio (MCR) as a Tool for Assessing the Value of Performing a Cumulative Risk Assessment Int. J. Environ. Res. Public Health 8, 2212-2225; doi: 10.3390/ijerph8062212 (http://www.mdpi.com/1660-4601/8/6/2212/)
6. Han X and Price P. 2011. Determining the Maximum Cumulative Ratios for Mixtures Observed in Ground Water Wells Used as Drinking Water Supplies in the United State Int. J. Environ. Res. Public Health 2011, 8(12), 4729-4745; doi:10.3390/ijerph8124729 (http://www.mdpi.com/1660-4601/8/12/4729/)
7. Hinderliter P, Price P, Bartels M, Timchalk C, Poet TS. 2011. Development of a source-to-outcome model for dietary exposures to insecticide residues: An example using chlorpyrifos. Regul Toxicol Pharmacol. *Volume 61, Issue 1*, *October 2011*, *Pages 82-92*
8. Price P, Schnelle K, Cleveland C, Bartels M, Hinderliter P, Timchalk C, Poet T. 2011. Application of a source-to-outcome model for the assessment of health impacts from dietary exposures to insecticide residues. Regul Toxicol Pharmacol. *Volume 61, Issue 1*, *Pages 23-31.*
9. 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, Zaleski R. 2011. Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment. Crit Rev Toxicol. 2011 Feb 10.
10. Rhomberg L, Goodman J, Haber L, Dourson M, Andersen M, Klaunig J, Meek B, Price P, McClellan R, Cohen S. 2011. Linear low-dose extrapolation for noncancer heath effects is the exception, not the rule. Crit Rev Toxicol. Jan;41(1):1-19.
11. Arnold S, Price P, Dryzga M. 2010. Defining the contribution of non-benzene sources of benzene metabolites in urine: implications for biomonitoring and

risk assessment. Chem Biol Interact. 2010 Mar 19;184(1-2):299-301. Epub Jan 21.
12. Price P. 2010. Synergy a Risk Management Perspective. In The *Principles and Practice of Mixtures Toxicology.* Wiley-VCH. Ed. Moiz Mumtaz.
13. Price P, Hollnagel H, Zabik J. 2009. Characterizing the noncancer toxicity of mixtures using concepts from the TTC and quantitative models of uncertainty in mixture toxicity. Risk Anal. Nov; 29 (11) :1534-48.
14. Price P, Wiltshire G. Modelling. 2009. the chronic non-cancer effects of mixtures of migrants using Cramer classes and quantitative models of uncertainty. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. Dec; 26 (12) :1547-55.
15. Price P, Keenan R, Swartout J. 2008. Characterizing interspecies uncertainty using data from studies of anti-neoplastic agents in animals and humans. Tox Appl Pharm Apr 16
16. Price P. and Jayjock M. 2008 Available data on naphthalene exposures: Strengths and limitations Reg Tox & Pharm Volume 51, Issue 2, Supplement 1, July, Pg 15-21
17. Griego F, Bogen K, Price P, and Weed D. 2008.Exposure, epidemiology and human cancer incidence of naphthalene. Reg Tox and Pharm 51(2), Supplement 1, July, Pages 22-26
18. Arnold S, Price P, 2007. Modelling Mixtures Resulting from Concurrent Exposures to Multiple Sources, Toxicology and Applied Pharmacology Volume 223, Issue 2, September , Pages 121-124
19. Koontz M, Price P, Hamilton J, Daggett D, Sielken R, Bretzlaff R, Tyler T. 2006. Modelling aggregate exposures to glycol ethers from use of commercial floor products. Int. J Toxicol. Mar-Apr; 25(2):95-107.
20. Price P, Mathis E, Tedder D, Chaisson C, Jayjock M. 2005. Human-effects Effectiveness Risk Characterization Model: Software for Simulating Human-Weapon Interaction with a Graphic Interface. *NATO Advanced Workshop Integrating Human Effectiveness and Risk Characterization of Non-Lethal Weapons*
21. Price P and Chaisson C. 2005. A Conceptual Framework for Modelling Aggregate and Cumulative Exposures to Chemicals, Journal of Exposure Analysis and Environmental Epidemiology 15, 473–481
22. Price P, Conolly R, Chaisson C, Young J, Mathis E, Tedder D. 2003. Modelling Inter-individual Variation in Physiological Factors Used in PBPK Models of Humans, Critical Reviews in Toxicology Vol. 33, (5): 469-503
23. Dourson M, Price P, Unrine J. 2002 Health Risks from Eating Contaminated Fish Comments on Toxicology Vol. 8, Num 4-6 Pg. 399-419
24. Wilson N, Craven V, Price P, Paustenbach D. 2002. Analysis of Possible Health Risks to Recreational Fishers Due to Ingesting DDT and PCBs in Fish from Palos

Verdes Shelf and Cabrillo Pier, Chapter 17, in *Human and Ecological Risk Assessment: Theory and Practice,* Ed. D. Paustenbach

25. Price P, Young J, Chaisson C. 2001. Assessing Aggregate and Cumulative Pesticide Risks Using A Probabilistic Model, Annals of Occupational Hygiene, *Vol 45 No. 1001, S131-S142*
26. Wilson N, Price P, Paustenbach D. 2001. An Event-by Event Probabilistic Methodology for Assessing the Health Risks of Persistent Chemicals in Fish: A Case Study at the Palos Verdes Shelf, Journal of Toxicology and Environmental Health, *62:595-642*
27. Price, P., Ginevan M., Barry, T., 2001. Qualitative and Quantitative Uncertainty Analysis, Chapter 12. *Residential Exposure Assessment*
28. Price, P.S. Chaisson C.F., Young, J.S., Christensen, C., Doyle E., Suhre, F.B., 1999. Background Document for the Session: Review of an Aggregate Exposure Assessment Tool FIFRA Scientific Advisory Panel, The LifeLine™ Project to Model Aggregate Exposures to Pesticides, Arlington, VA, September 22
29. Price, P.S., Keenan, R.E., Schwab B. 1999. Defining the Interindividual (Intraspecies) Uncertainty Factor. HERA, 5(5) p. 1023-1033
30. Carlson-Lynch, H., P.S. Price, J.C. Swartout, M.L. Dourson, and R.E. Keenan. 1999. Application of quantitative information on the uncertainty in the RfD to noncarcinogenic risk assessments. HERA 5(3) p. 527-547.
31. Muir, W.R., Young, J.S., Benes, C., Chaisson, C.F., Waylett, D.K., Hawley, M.E., Sandusky, C.B., Sert, Y., DeGraff, E., Price, P.S., Keenan, R.E., Rothrock, J.A., Bonnevie, N.L., McCrodden-Hamblin, J.I., 1998. A Case Study and Presentation of Relevant Issues on Aggregate Exposure, in Aggregate Exposure a Report to EPA/ ILSI Workshop, Ed. Olin, S. ILSI Press, Washington DC.
32. Price, P.S., P. K. Scott, N. D. Wilson, D.J. Paustenbach. 1998 An Empirical Approach for Deriving Information on Total Duration of Exposure from Information on Historical Exposures, *Risk Anal.* (18) 4
33. Swartout, J.C., P.S. Price, M.L. Dourson, H. Carlson-Lynch, and R.E. Keenan. 1998. A probabilistic framework for the reference dose, *Risk Anal.* (18) 3, 271-282
34. Price, P.S., R.E. Keenan, J.C. Swartout, C.A. Gillis, H. Carlson-Lynch, and M.L. Dourson. 1997. An approach for modelling noncancer dose responses with an emphases on uncertainty. *Risk Anal.* Vol 17, No. 4.
35. P.S. Price and Stickney, J.A. 1997. The role of statistics, policy, and management issues in the decision to adopt linear non-threshold dose response models. *Comm. Toxicol.*, 6(2): 139-149.
36. Price, P.S., C.L. Curry, P.E. Goodrum, M.N. Gray, J.I. McCrodden, N.W. Harrington, H. Carlson-Lynch, and R. E. Keenan. 1996. Monte Carlo modelling of time-dependent exposures using a Microexposure event approach. *Risk Anal.* 16(3): 339-348.

37. Price, P.S., S.H. Su, J.R. Harrington, and R.E. Keenan. 1996. Uncertainty and variation in indirect exposure assessments: An analysis of exposure to tetrachlorodibenzo-*p*-dioxin from a beef consumption pathway. *Risk Anal.* 16(2): 263-277.
38. Curry, C.L., P.S. Price, D.G. Gunster, N.L. Bonnevie, and T. B. Abel. 1995. Interlake Variation in Polychlorinated Biphenyl Bioaccumulation Factors for the Great Lakes, Water Environment Federation, Annual meeting, May
39. Harrington, N.W., C.L. Curry, H.J. Carlson-Lynch, and P.S. Price. 1995. The Microexposure Event Modelling Approach to Probabilistic Exposure Assessment. Air and Waste Management Annual Meeting, San Antonio. Manuscript A443. May.
40. Ebert, E.S., P.S. Price, and R.E. Keenan. 1994. Selection of fish consumption estimates for use in the regulatory process. *J. Exp. Anal. Environ. Epid.* 4(3):373-394
41. Finley, B., D. Proctor, P. Scott, P. Price, N. Harrington, and D. Paustenbach. 1994. Recommended distributions for exposure factors frequently used in health risk assessment. *Risk Anal.* 14(4):533-553
42. Keenan, R.E., B.L. Finley, and P.S. Price. 1994. Exposure assessment: then, now, and quantum leaps in the future. *Risk Anal.* 14(3):225-230.
43. Price, P.S., S.H. Su, and M.N. Gray. 1994. The effect of sampling bias on estimates of angler consumption rates in creel surveys. *J. Exp. Anal. Environ. Epid*. 4(2):355-372
44. Paustenbach, D.J., R. Bass, and P. Price. 1993. Benzene toxicity and risk assessment (1972-1992): Implications for future regulations. *Environ. Health Perspect.*
45. Sherer, R.A. and P.S. Price. 1993. The effect of cooking processes on PCB levels in edible fish tissue. *Qual. Assur: Good Practice, Reg. and Law* 2(4):396-407.
46. Paustenbach, D.J., P.S. Price, W. Ollison, C. Blank, J. Jernigan, R. Bass, and H.D. Peterson. 1992. Reevaluation of benzene exposure for the Pliofilm (rubberworker) cohort (1936-1976). *J. Toxicol. Environ. Health* (36):177-231.
47. Price, P., J. Sample, and R. Strieter. 1991. PSEM a model of long term exposures to emissions of point sources. In: *Proceedings of the 84th Annual Meeting of Air and Waste Management Association.* June.
48. Price, P., J. Sample, and R. Strieter. 1991. Determination of less-than-lifetime exposures to point source emissions. *Risk Anal*. 12(3):367-382.
49. Price, P. 1988. Apportionment of reference doses. In: *Proceedings of the Society for Risk Analysis.*
50. Price, P. 1984. Acceptable levels of risk and standards. In: *Proceedings of the Society for Risk Analysis.*
51. Allman, J. and P. Price. 1980. A synopsis of perspectives on the innovative and alternative technology program. Innovative and Alternative Treatment Technology program. *J. Wat. Poll. Contrl. Fed.*