José A. Torres · Sol Bobst *Editors*

Toxicological Risk Assessment for Beginners

Springer

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I dedicate this book to my wife. My personal proof that Angels walk among us.
I love you, Nilsa Rivera-Del Valle.

José Anibal Torres-Hernández

I dedicate this book to my father;

Steve W. Bobst, MS SPHR
1944–2013;

my best friend, I am the man I am because of his love and support. I miss him everyday.

Sol Mio Bobst

Foreword

"Risk assessment is easy. You can learn it in two steps…. Each step takes 10 years." This quote, attributed to Arnold Lehman of the U.S. Food and Drug Agency in the early 1950's, epitomizes the way many of us risk assessment scientists learned our trade. That is, the very slow accumulation of knowledge wrought not only from the daily practice of one of risk assessment's many disciplines, but also from the rubbing of shoulders with other disciplines to develop a judgment or decision for a particular chemical or situation. The interactions among the many disciplines, such as toxicology, epidemiology, and mathematics, more often resembled a logic problem or perhaps a Chinese puzzle. It was seldom that pulling on only one aspect of the problem, or depending on only one discipline among several, yielded the best decision. It was the integration of many aspects and disciplines that often yielded the best solutions. Those of us fortunate enough to have a good mentor or two, perhaps took these two steps a little more quickly. But even after 30 years in the field, I learn new things each week, and do not for one moment think it is different with any of my colleagues.

Periodic attempts at expediting this learning process have been made through the publication of books, and the development of numerous risk assessment guidelines by federal agencies of many countries or international groups, such as the International Programme on Chemical Safety (IPCS). These guidelines have in particular codified best practices. Unfortunately, these books and guidelines were written more for the practicing risk assessment scientist, not the novice.

Quite simply, a need exists for basic level and accessible educative materials in risk assessment field, and, thus, the motivation for this book. What you will find here is a focus on graduate level education, but with plenty of substance for post-doctoral students and experts from other fields that are beginners in risk assessment. The overall emphasis of this book is on balancing the theory of risk assessment with its practice. In fact, one needs to have a fundamental grasp of the overall theory of risk assessment, and be grounded in one of its many disciplines, in order to make a good practitioner.

Written by expert risk assessment scientists, who nevertheless are still learning themselves, this book will encompass the traditional areas of hazard identification–both toxicology and epidemiology evaluation, dose-response assessment, exposure

assessment, risk characterization, and mixtures assessment. However, this book also delves into emerging areas, such as data from genomic arrays, the European Union REACH, global harmonization of risk assessment approaches, training boot camps, and case studies.

In nearly all cases, risk assessment decisions and judgments will need to be made in the face of uncertainty, whether it be with individual data, extrapolation to a more relevant, and usually lower, exposures, or with the use of the results of one animal specie as a surrogate for the anticipated results in another specie. In many of these cases, the uncertainties are large and the resulting risk assessment values would perhaps be more appropriately associated by a range, rather than a single value. In fact, the idea of using a range in risk assessment values to represent the underlying biological variability has currency in recent U.S. National Academy of Science findings. But this idea really is not new. In fact, "It is the mark of an instructed mind to rest satisfied with the degree of precision which the nature of the subject permits and not to seek an exactness where only an approximation of the truth is possible." Aristotle

Sincerely,

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Editors' Preface

We are equally passionate about the subject of risk assessment and enjoyed working together to design, propose and edit a book to convey our ideas for a simple reason—no one to date has published a book targeting individuals that want to learn about risk assessment, but have no experience in it. While there are several expert level books, they aren't for beginners.

Sol noticed when he was starting to study risk assessment, that there weren't any books like this. He used materials from publications, and several professional societies to get educated. Jose participated in several workshops on risk assessment, but did not find a basic book to complement his training. Jose also noticed that educational activities at a national meeting were not focusing on graduate students or post-doctoral trainees but rather expert practitioners. He thought there was a great opportunity to provide risk assessment education, thus Jose decided to conduct a skills survey and developed a panel presented at Society of Toxicology.

After that, Springer approached us with the idea of a book. The journey from idea to publication has taken us approximately two years, and every bit of the effort has been worth it. Sol and Jose have spent several Mondays and Fridays on conference calls, reviewing and discussing the components of the book to make sure introductory concepts are present in each chapter. The goal is by no means to be comprehensive, but rather to function as a bridge and connect readers that we presume to have a toxicology preparation, with the practical aspects of risk assessment.

Each chapter is composed of four parts that include: an abstract, learning objectives, main body and a short summary. We recommended reading the chapters in the order presented as each chapter creates a foundation for the following chapter. Learning a new topic should cover two basic questions. The first question is what? This book breaks down the "what" of risk assessment in building block chapters. The second question is how? This book also shows "how" through several examples within text, as well as case studies to practice at the end.

We are excited about this contribution to the scientific and professional community, we hope that you find it a useful resource, whether you are using it in a class, or you are learning risk assessment on your own.

We welcome your feedback on the book, to help us improve future editions, please contact us at smbobst@yahoo.com or joseanibaltorres@gmail.com.

Best Regards,

Sol Bobst, Ph.D., DABT.

José A. Torres, M.S., Ph.D.

Acknowledgements

First and foremost the editors want to recognize each single chapter author that gracefully volunteered his/her time and expertise in the subject of Risk Assessment to create a book suitable for beginners. It is because of their shared common goal to help and educated the next generation of risk assessors that they made this book a reality. Thank you all. Furthermore, a special appreciation note goes to Manika Power, our Springer Editor, and Rosie Daniel, our project assistant at Springer. Thank you both for trusting, believing and support our pursuit of this project. Without you this book would be only an idea. Finally to Lois Ricciardi, thank you for editing and proofreading Chapters 1 & 11 as your comments improved the chapter's quality and flow. In closure, much appreciation to the graduate students and post-doctoral trainees, and all professionals that are eager to learn about the application of Toxicology in Risk Assessment. Thank you, as for your interest and benefit is the primordial motivation this work was created. The editors desire for the readers to find this book of value and enjoy learning and understanding a challenging subject. Please if you find this book of any help or believe to have a constructive criticism for future versions do tell us by email to: smbobst@yahoo.com or joseanibaltorres@gmail.com. Now let our fun begin!

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Editors

José A. Torres, M.S., Ph.D. has experience in risk assessment, project management, quality control, quality auditing, and quality assurance as applied to consulting, pharmaceuticals and the medical device industry. His interest in risk assessment drove him to pursue advanced courses and workshops at Harvard School of Public Health for Analyzing Risk, TERA Dose-Response Boot Camp, and PBPK basic modeling. He completed a Bachelor in Biology (*Magna Cum Laude*) with specialty in Biomedical Sciences from Inter American University of Puerto Rico; a Masters in Environmental Science & Management from Turabo University at Puerto Rico; and a Doctoral degree in Environmental Toxicology, sponsored by NASA, from Texas Southern University, Houston Texas.

Sol Bobst, Ph.D., DABT has participated in several risk assessment reviews, including a publication in the Regulatory Toxicology and Pharmacology journal. He is a ten year member of the Society of Toxicology, and the past president of the Ethical, Legal, and Social Issues specialty section. He graduated *Magna Cum Laude, University Honors* with a Bachelor's Degree in Chemistry from Drake University in Des Moines, IA. His Doctoral degree is from the University of Texas Health Science Center at Houston, Texas, and he holds a board certification from the American Board of Toxicology. He continues to live and work in Houston, Texas.

List of Abbreviations

ABNT	Associação Brasileira de Normas Técnicas
ACGIH	American Conference Of Governmental Industrial Hygienists
ACToR	Aggregated Computational Toxicology Online Resource
ADHE	Ability To Detect Health Effects
ADIs	Acceptable Daily Intakes
ADME	Absorption, Distribution, Metabolism And Elimination
AED	Aerodynamic Equivalent Diameter
AEGLs	Acute Exposure Guideline Levels
AFs	Assessment Factors
AIHA	Industrial Hygiene Association
AL	Acceptable Levels
ALs	All Scaling Factors
AMAHE	Ability To Mitigate Adverse Health Effects
AML	Acute Myelogenous Leukemia
ANLL	Acute Nonlymphocytic Leukemia
ANVISA	Brazilian Health Surveillance Agency
AOELs	Acceptable Operator Exposure Levels
AOP	Adverse Outcome Pathway
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARA	Alliance For Risk Assessment
ATSDR	Agency For Toxic Substance And Disease Registry
BEEL	Biological Environmental Exposure Level
BEIs	Biological Exposure Indices
BID	Interamerican Bank of Development
BMC	Benchmark Concentration
BMCL	Benchmark Concentration Lower-Confidence Limit
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower-Confidence Limit
BMDS	Benchmark Dose Response/Software Program
BMR	Benchmark Response
BW	Body Weight
CAN	Andean Community

CBRA	Community-Based Risk Assessment
CCID	Chemical Classification and Information Database
CDC	Center For Disease Control
CDR	Crude Death Rate
CEPA	Canadian Environmental Protection Act
CERC	Columbia Environmental Research Center
ChemSTEER	Chemical Screening Tool For Exposures And Environmental Releases
CI	Confidence Interval
CICLOPLAFEST	Comisión Intersecretarial para el Control del Proceso y Uso de Plaguicidas, Fertilizantes y Sustancias Tóxicas
CLL	Chronic Lymphocytic Leukemia
CLP	Regulation No 1272/2008
C_m	Mixture Concentration Expressed As The Index Chemical
CML	Chronic Myelogenous Leukemia
CNS	Central Nervous System
COAG	Council of Australian Governments
COSHH	Control Of Substances Hazardous To Health
CPF	Cancer Potency Factor
CRA	Cumulative Risk Assessment
CRARM	Congressional Presidential Commission On Risk Assessment And Risk Management
CRC	Chemical Registration Center
CRO	Contract Research Organization
Crow	Members Of The Apsaálooke (Crow) Tribe In Montana
CSA	Chemical Safety Assessment
CSAFs	Chemical Specific Adjustments Factors
CSD	Cause-Specific Death Rate
CSS	Chemical Safety for Sustainability
CVD	Cardiovascular Disease
DALYs	Disability-Adjusted Life Years
DBP	Disinfection Byproducts
DCR	Death-To-Case Ratio
DMAV	Dimethylarsinic Acid
DMELs	Derived Minimal Effect Levels
DNEL	Derived No Effect Levels
DPD	Dangerous Preparations Directive
DSD	Dangerous Substances Directive
DSL	Domestic Substance List
DTSC	California Department Of Toxic Substances And Control
DW	Disability-Weighting Factor
EASE	Estimation And Assessment Of Substance Exposure
EBT	Evidence Based Toxicology
EC	European Commission
EC_{50}	Effective Concentration At 50 %

ECC	European Economic Community
ECDC	European Centre For Disease Prevention And Control
ECETOC	European Center For Ecotoxicological And Toxicology Of Chemicals
ECHA	European Chemicals Agency
ED_{10}	Effective Dose At 10%
ED_{50}	Effective Dose At 50%
EDSP	Endocrine Disruptor Screening Program
EEA	European Environment Agency
EFSA	European Food Safety Authority
EINECS	European Inventory of Existing Commercial Substances
EMA	European Medicines Agency
EMKG-Expo	Easy-To-Use Workplace Control Scheme For Hazardous Substances
EPA	Environmental Protection Agency
ERA or ECOTOX	Ecological Risk Assessment
ERPGs	Emergency Response Planning Guidelines
ES	Exposure Scenario
ESR	Existing Substances Regulation
ETSP	European Society Of Toxicologic Pathology
EU	European Union
FDA	Food And Drug Administration
FDCA	Food, Drug, And Cosmetic Act
FID	Flame Ionization Detectors
FQPA	Food Quality Protection Act
GHS	United Nations Globally Harmonized System Of Classification And Labeling
GLP	Good Laboratory Practices
GX	Glycol Derivative
HAWC	Health Assessment Workspace Collaborative
HED	Human Equivalent Dose
HHRA	Human Health Risk Assessment
HIint	Interaction-Weighted Hazard Index
HQ	Hazard Quotients
HQ	Hydroquinone (Epidemiology Chapter)
HSDB	Hazardous Substance Database
HSE	U.K. Health & Safety Executive
HSL	U.K. Health & Safety Laboratory
HSNO	Hazardous Substances and New Organisms
HtLF	High To Low Dose Risk Extrapolation Factor
HTS	High Throughput Screening
IARC	International Agency For Research On Cancer
ICER	Incremental Cost Effectiveness Ratio
IDLH	Immediately Dangerous To Life Or Health
IMR	Infant Mortality Rate

IPCS	International Programme On Chemical Safety
IR	Infrared Spectrometers
IRIS	Integrated Risk Information System
ITER	International Toxicity Estimates For Risk Databases
K-REACH	Korea-REACH
LARAW	Latin American Risk Assessment Workshop
LC_{50}	Lethal Concentration At 50%
LD_{50}	Lethal Dose At 50%
LEV	Local Exhaust Ventilation
LOAEC	Lowest Observed Adverse Effect Concentration
LOAETL	Lowest Observable Adverse Effect Level
LOEL	Lowest Observable Effect Level
MDA	Methylenedianiline
MDS	Myelodysplastic Syndromes
MEP	Ministry of Environmental Protection
MERCOSUR	Treaty of Asunción, an economic and political agreement
MIE	Molecular Initiating Event
MOA	Mode-Of-Action
MOE or MoE	Margin Of Exposure
MPD	Myeloproliferative Disorders
MTD	Maximum Tolerated Dose
NAS	National Academy Of Science
NCEA	The National Center for Environmental Assessment
NCI	National Cancer Institute
NHANES	The National Health and Nutrition Examination Survey
NHL	Non-Hodgkin Lymphoma
NICNAS	National Industrial Chemicals Notification And Assessment Scheme Chemical Assessment Reports
NIOSH	National Institution Of Occupational Safety And Health
NOAEL	No Observable Adverse Effect Level
NOEL	No Observable Effect Level
NRC	National Research Council
NTP	National Toxicology Program
NZIoC	New Zealand Inventory of Chemicals
OCs	Operational Conditions Of Use
OECD	Organisation For Economic Co-Operation And Development
OEHHA	California Office Of Environmental Health And Hazard Assessment
OELs	Occupational Exposure Limits
OPPT	Office Of Pollution Prevention And Toxics
OR	Odds Ratio
OSHA	Occupational Safety And Health Administration
PBBs	Polybrominated Biphenyls
PBDEs	Polybrominated Diphenyl Ethers
PBPK	Physiologically Based Pharmacokinetics

PC	Personal Controllability
PELs	Permissible Exposure Limits
PICCS	Inventory of Chemicals and Chemical Substances
PID	Photoionization Detectors
PMR	Proportionate Mortality Ratio
POD	Point Of Departure
PP	Precautionary Principle
PPB	Parts Per Billion
PPE	Personal Protective Equipment
ppm	Parts Per Million
PPRTVs	Provisional Peer Reviewed Toxicity Values
PROCs	Process Categories
Prop 65	The Safe Drinking Water And Toxic Enforcement Act Of 1986
PVC	Polyvinyl Chloride
q	Chromosome Long Arm
QALYs	Quality Adjusted Life-Years
QSARs	Quantitative Structural-Activity Relationship
r	Correlation
RA	Refractory Anemia (Epidemiology Chapter)
RA	Risk Assessment
RAEB	Refractory Anemia With Excess Blast
RAGS	Risk Assessment Guidance For Superfund
RAR	Risk Assessment Report
RARs	EU Risk Assessment Reports
RASS	Risk Assessment Specialty Section
RCR	Risk Characterization Ratio
REACH	Registration, Evaluation, Authorisation And Restriction Of Chemicals
Red Book	Risk Assessment In The Federal Government: Managing The Process
RfC	Reference Concentration
RfD	Reference Dose
RI	Risk Index
RiskIE	Risk Information Exchange
RITE	Risk Is Dependent On Toxicity And Exposure
RMMs	Risk Management Measures
RPF	Relative Potency Factor
RQ	Risk Quotients
RR	Relative Risk
RTECS	Registry Of Toxic Effects Of Chemical Substances
S.D.	Standard Deviation
SAGARPA	Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food
SAICM	Strategic Approach to International Chemicals Management
SAR	Structure Activity Relationship
SAWS	State Administration of Work Safety
SCCS	Scientific Committee on Consumer Safety

SCENIHR	Scientific Committee On Emerging And Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SCOEL	Scientific Committee On Occupational Exposure Limits
SCP	California Safer Consumer Products Regulation
SCT	Secretariat of Communications and Transport
SDS	Safety Data Sheets
SDWA	Safe Drinking Water Act
SE	Secretariat of the Economy
SEGs	Similar Exposure Groups
SEMARNAT	Secretariat of the Environment and Natural Resources
SES	Socioeconomic Status
SG	Standard Gambling
SIDS	Screening Information Data Set
Silver Book	Science And Decision: Advancing Risk Assessment
SMR	Standardized Mortality Ratio
SOT	Society Of Toxicology
SSA	Secretaría de Salud (Health Secretariat)
STELs	Short-Term Exposure Limits
STPS	Secretariat of Labor and Social Welfare
TASE	Target Animal Safety Evaluation
TB	Tubercle bacillus
TCCR	Transparency, Clarity, Consistency, And Reasonableness
$TC_{Lo,}$	Lowest Published Toxic Concentration
TD	Tumorigenic Dose
TDI	Tolerable Daily Intake
TD_{Lo}	Lowest Published Toxic Dose
TEELs	Temporary Emergency Exposure Limits
TEF	Toxic Equivalency Factor
TERA	Toxicology Excellence For Risk Assessment
TGD	Technical Guidance Document on Risk Assessment
TLV	Threshold Limit Value
TOX	Total Organic Halide
ToxCast	Toxicology Forecaster Tool
TOXNET	Toxicology Data Network
ToxRefDB	Toxicity Reference Database
ToxRTool	Toxicological Data Reliability Assessment Tool
TRA	Targeted Risk Assessment
TSCA	Toxic Substances Control Act
TSCATS	Toxic Substance Control Act Test Submission Database
TTC	Threshold Of Toxicological Concern
TTD	Target Organ Toxicity Doses
TTO	Time-Trade-Off
TWA	Time-Weighted Average
TWA-REL	Time-Weighted Average Recommended Exposure Limits

UF	Uncertainty Factors
USDA	United States Department Of Agriculture
USGS	Unites States Geological Survey
UV	Utility Value
VAS	Visual Analogue Scale
WEELs	Workplace Environmental Exposure Levels
WHO	World Health Organization
WOE	Weight Of Evidence
YLD	Years Lost Due To Disability
YLL	Years Of Life Lost Due To Premature Mortality

Chapter 1
Introduction

José A. Torres and Sol Bobst

"Knowing is not enough; we must apply. Willing is not enough; we must do." Johann Wolfgang von Goethe.

Abstract Risk Assessment is a multi-step process used by professionals to make decisions for "safe" use of chemicals in commercial, industrial, and environmental settings. The process includes Hazard Identification, Dose Response Assessment, Exposure Assessment, and Risk Characterization. Risk Assessment is an evolving field influenced by technological approaches and advancement. The history and basic tenets of Risk Assessment are introduced in this chapter to prepare the reader for the rest of the book.

Keywords Risk · Assessment · Red Book · Silver Book · Stressor · Exposure · Effect · Endpoint · Weight of evidence · WOE · HHRA · ERA · CRA · Integrated framework · Hazard identification · Dose-response · Exposure assessment · Risk characterization

Student Learning Objectives

- Introduce basic risk assessment concepts and steps
- Familiarize with important terminology

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- Identified risk assessment importance in protection
- To recognize the traditional and integrated frameworks

Introduction

Many professionals from a variety of disciplines often face the challenge of answering the question "How do I make a decision with scientific data, in order to protect the health and safety of my stakeholders, while still allowing use of the substance?" To answer this question, the professional has to understand the definition and evaluation of risk. Most academic scientific training focuses on hypothesis driven approaches. The practice of risk assessment is a topic not traditionally taught in most academic settings but rather learned on the job under an experienced risk assessor. However, lately more academic institutions are incorporating courses on risk assessment in their programs. The authors of this book provide the readers with the foundations of risk assessment as it applies to human health data. Throughout the book, the authors also provide case studies and examples to demonstrate the practice with the explanation of theory. This chapter discusses the history and current state of risk assessment from a general point of view to prepare the reader for the upcoming chapters that cover the material in depth.

Human Health Risk Assessment (HHRA) Steps

The information of HHRA steps below is not intended to be comprehensive. It serves as a brief introduction to ease the reader in the topic before moving into the HHRA chapters for a more in depth discussion of each step. The basic HHRA process that will be covered in detail in this book includes: (a) Hazard Identification, (b) Dose Response Assessment, (c) Exposure Assessment and (d) Risk Characterization. Paustenbach (2002) provided the main points and summarized key minimal components for each step. In hazard identification the minimal key components are (a) what is the key toxicological study that provides the basis for health concerns; (b) are there other health endpoints of concern; (c) is there available epidemiological or clinical data; (d) how much is known about how the chemical produces adverse effects; (e) is there any non-positive data in animals or people; and (f) what are the conclusions of hazard identification? The key and minimal components for dose-response are (a) what data was used to develop the dose response curve; (b) what model was used to develop the dose-response curve; and (c) what is the route and level of exposure observed as compared to expected human exposure? The minimal key components for exposure assessment are (a) what are the most significant sources of environmental exposures; (b) what population was assessed; (c) what was the basis for the exposure assessment; (d) what are the key descriptors of risk; (e) is there any reason to be concerned about cumulative or multiple exposures;

and (f) what are the conclusions of the exposure assessment? Risk characterization (EPA Elements of Risk Characterization 1995) is a summary of key issues and conclusions of the previous components of the risk assessment, i.e., (a) what is the overall picture of risk, based on hazard identification, dose response and exposure assessment; (b) what are the major conclusions, strengths, limitations, uncertainties and variabilities; (c) are there other viable options and if so, how do they compare in risk; and (d) how does the risk compare to past or similar risk assessments and describe any significant difference?

The hazard identification step determines if the agent in question causes adverse effects. An excellent example of this step is the use of the United Nations Globally Harmonized System of Classification and Labeling, or (GHS). One way of defining risk is to treat it as a two-part equation. Risk = Hazard × Exposure. Put in simpler terms, Risk = (How Bad?) × (How Much?). In order to address the question of "How Bad" scientific and regulatory communities have developed schemes to address the "levels" of hazards, based on experimental outcomes. Hazards can be either acute or chronic, and they can have a relatively low or high ranking. For hazard communication and labeling requirements of chemicals, the level of the hazard is tied to the safeguard measures provided. Hazard identification is also useful for guiding and planning testing strategies that will be used in risk assessment evaluations. To ensure that the process of hazard identification provides a qualitative and consistent foundation of a risk assessment, certain guidelines are applied to the Hazard Identification and Characterization process. This information is covered in more detail in the hazard identification chapter.

The dose response step asks what are the levels of exposure and the extent of severity of health effects that can occur? The EPA separates dose-response into threshold (non-cancer) and non-threshold (cancer) concepts. The threshold (non-cancer) method includes point of departures such as NOEL (No observable effect level), LOEL (Lowest observable effect level), NOAEL (No observable adverse effect level), and LOAEL (Lowest observable adverse effect level). The EPA uses safety factors such as: human variability, animal to human extrapolation, sub-chronic to long-term exposure extrapolation, LOAEL to NOAEL, and modifying factors to calculate a reference dose or reference concentration (RfD or RfC). This terminology (RfD or RfC) indicates the concentration of a chemical at which it is likely to be without appreciable risk during a lifetime. The no-threshold (cancer) method uses weight of evidence (WOE) and determination of cancer potency factors. The dose-response chapter will cover the concepts of threshold and non-threshold in more detail.

The exposure assessment step involves recognition of the exposure setting, identification of exposure pathways and quantification of the exposures. Exposure may be estimated with direct measurement, indirect estimation or exposure reconstruction. The exposure setting helps to identify point or non-point sources of contamination, physical setting and population exposed. The exposure pathways may include ground water, surface water, air, soil, food and occupational settings. The quantification for exposure uses prediction of frequency and severity of effects in exposed populations. Further exposure assessment may benefit from biological and

statistical knowledge, biomonitoring, scenario evaluation and considerations of uncertainties. Exposure assessment is a complex subject and an in depth understanding of how human exposure data is collected, analyzed and presented in the *context of occupational* risk assessment is provided in the exposure assessment chapter.

The risk characterization step uses the data obtained from hazard identification, dose-response, exposure data, variability, and uncertainties to reach a systematic, scientific and valid conclusion about the risk in question. This may be the most complicated step in the risk assessment process and requires significant expertise and knowledge. The purpose of risk characterization is to answer the following questions: Is there a health risk? What is the magnitude? How well is it known? To answer these questions, a summary of key findings, including uncertainties and variability, from the other three steps mentioned above is included in the risk characterization process. This compilation of data should attempt to interpret the information for a broader audience and should provide a statement about the risk in question. The risk characterization chapter covers this complex subject in greater depth.

Risk management is the process of decision-making about the issue of concern (e.g., chemical, physical, biological risk). Normally it is a regulatory agency such as EPA, FDA, and OSHA, or in some cases a court of law, that determines important decisions about risk. Risk managers not only consider the risk assessment documentation, but also evaluate economical, social, political and public health information. They use this information to develop several possible outcomes or regulatory options and based upon their expertise, select what they believe to be the best option.

Definitions of Important Concepts

Words requiring definitions to build vocabulary are: risk, stressor, exposure, assessment and risk assessment. The EPA defines **risk** as the ***chance*** of harmful effects to human health or ecological systems resulting from exposure to an environmental stressor. A **stressor** is considered to be a chemical, physical or biological entity that can induce an adverse response or reaction. The stressor may affect natural resources or entire ecosystems (animals, plants, and the environment in which they interact). **Exposure** is a measure of the dose in humans or the concentration in an environmental matrix (air, water, soil) of a stressor to a biological or ecological system. Accurate characterization of exposure is essential for the risk assessment process as risk depends on the following factors: how much chemical or stressor is present in the matrix (air, water, soil, etc.), how much exposure the entity (individual person or ecological system) has with the chemical and the inherent toxicity of chemical. The Toxicology Education Foundation provides an easy mnemonic "**RITE**," meaning *Risk* is dependent on *Toxicity* and *Exposure* (Karmin 2011).

Assessment is defined as the act of making a judgment about something or the act of assessing something, or the evaluation or estimation of the nature, quality, or ability of someone or something. Thus, ***risk assessment*** *is the evaluation (or estimation) of the chance (likelihood) a harmful effect occurs to humans and/or*

ecological systems from exposure to a stressor. The risk assessment terminology may become confusing and cumbersome; case in point—the risk assessment outcome is different than the risk assessment process. Experts sometimes refer to risk assessment in reference to the actual product/outcome, i.e., document or report created. In other situations, they might refer to risk assessment as the process (hazard identification, dose-response, exposure assessment and risk characterization) or a specific part of the process.

A key distinction to note is the difference between hazard and risk. Often these two terms create confusion. **Hazard**, is defined as any source (chemical, physical, biological) of *potential damage*, harm or adverse health effect on something or someone under certain conditions. The word **risk** involves *chance or probability* that is dependent on the situation or condition under which the stressor is encountered. In the most simplistic view, risk is a question of chance and hazard is a question of potential (or capacity) to cause harm. To consolidate this information consider that a bus with faulty brakes is speeding toward a person standing at an intersection. Is the speeding bus a hazard or risk? The circumstance or source (bus speeding with faulty brakes toward a person) makes this a hazard. The answer in relation to risk is dependent on the bus distance—is the bus 10 sec (high risk), 2 min (medium risk), or 5 min (low risk) away from the person? A bus 5 min away will provide enough time for the person to move out of the intersection and thus away from danger compared to a time period of 10 sec. Therefore, the bus is a hazard (type of yes or no answer to the *potential to cause harm*), but the risk (*chances or likelihood*) of getting hit will vary depending on the specific scenario.

Although the last example was good for introducing the concept of hazard, unfortunately the hazard identification process is not that simple in real life. Scientists use a more complex qualitative and/or quantitative approach known as weight of evidence (WOE) to characterize hazard[1] (a step beyond hazard identification), where most, if not all, of the data available (positive and negative, mechanistic and non-mechanistic, *in vivo* and *in vitro*, human and animal) for a particular issue of interest where uncertainty is inherent to such issue is used to reach a conclusion. The WOE approach involves aggregating evidence (lines of evidence) from various modalities (human, animal, etc.), collecting or developing explanations (by assigning causality), assessing such explanations for consistency with evidence and reaching a conclusion (Krimsky 2005). Causality is a difficult term to define. The Merriam-Webster dictionary describes it as the relation between cause and effect. A more informative approach is defining cause in relation to a disease. Causality inference is explained in more detail in the epidemiology chapter. To this end the principles of rigor, completeness, transparency, scope and practicability are used to

[1] Hazard characterization is a term used in literature loosely with several meanings. For instance: (a) any step beyond simple (yes/no) type of answer that help describe the mechanisms involved in causing harm i.e. WOE, mode of action; (b) a combination of qualitative (hazard identification) and quantitative (dose-response) assessment; and (c) a summary of key results from hazard identification steps i.e. findings, issues, mode of action, strength and weakness, WOE, alternative explanations and susceptible population. The authors share this footnote to advise the readers of this possible variance of meaning.

see if the WOE conclusion regarding causality is well developed. WOE ranges from essentially qualitative methods in listing evidence and best professional judgment (professional opinions based on years of experience), to more quantitative methods such as logic and causal criteria, indexing and scoring, to purely quantitative methods of statistics and multi criteria decision analysis (Linkov 2009). Weed (2005) stated that WOE is used in the literature in three different forms (1) metaphorical to represent a summary or synthesis of a body of scientific evidence without any particular methodology; (2) methodological or developed under a particular qualitative or quantitative methods; and (3) theoretical as pattern recognition or evidentiary role in a court of law (where WOE adheres to the principle of relevance, reliability, sufficiency and standard of proof). The EPA first used WOE in 1986 in the guidelines for carcinogenic risk assessment and later updated in 2005 (Linkov 2009). The hazard identification chapter explains how the WOE approach is used to facilitate hazard characterization, but WOE is not an exclusive process for hazard characterization and might be used in other steps such as dose-response. WOE continues to be a topic for dialogue in the risk assessment community. For example, in the NRC (2014) report: "Review of EPA's Integrated Risk Information System (IRIS) Process" suggested that EPA use the term evidence integration rather than WOE.

The risk assessment process identifies and estimates risk, rather than hazard. Thus at some point, all the information collected, organized, analyzed and synthesized from the risk assessment process should be presented in terms of risk, a step normally accomplished in the risk characterization. The risk characterization chapter offers a collection of several formulas commonly used and necessary in the risk assessment process. Although not all formulas are used in every occasion, a familiarity of how risk statements are presented is important for the newcomer.

Additional definitions of value to the reader are effect, adaptive effects, compensatory effects, critical effect, adverse effect, mode of action, point of departure and endpoints. These terms will be used throughout this book and an early presentation should prepare the reader. **Effect** is any change positive or negative that occurs and is observed, quantified and/or measured. As scientific understanding of specific effects evolve, a range (or degree) of effects is used to improve precision and understanding in risk assessment. **Adaptive effects** are changes occurring in an organism (or cell) in response to a xenobiotic in which the organism (or cell) manages and tolerates survival in the presence of the new environment (containing xenobiotic) without loss of function (Keller 2012). The characteristics of adaptive effects are that effects are reversible, effects have an enhanced capacity to respond to stress and thus potentially provide a beneficial effect on function or structure, and effects do not compromise viability at all levels of tissue organization (Williams and Iatropoulos 2002). A **compensatory effect** is when the organism no longer manages to balance the changes without further involvement and attempts to counterbalance the undesirable effect by altering its system in some way so that the net result is to nullify or minimize changes. A **critical effect** is when multiple effects are present due to one or more stressors. After analyzing dose-response data, the effect observed at the lowest dose that shows an effect is considered the critical effect. **Adverse effect** is specifically referring to changes that are considered negative. Dourson et al. (2013)

describes in more detail the degree of continuum effects. **Mode of action** (MOA) is a term used to describe the most important or relevant steps regarding a stressor outcome when the complete mechanisms of action are not well known or understood. The EPA defines **point-of-departure** (PoD) as *a dose that can be considered to be in the range of observed responses, without significant extrapolation. A PoD can be a data point or an estimated point that is derived from observed dose-response data. A PoD is used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures.* The EPA defines **endpoints** as an observable or measurable biological or chemical event used as an index of the effect of a stressor on a cell, tissue, organ, organism, etc. These terms are covered in subsequent chapters with proper context.

History and Evolution of Risk Assessment

Risk assessment is the characterization of potential adverse health effects resulting from human and ecological exposure to environmental hazards. The EPA selected definition for risk assessment was obtained from a Publication by the National Research Council in 1983 (NRC 1983), "Risk Assessment in the Federal Government: Managing the Process" (commonly called "the Red Book" due to its red cover) that offers the first seminal treatment of the risk assessment process -*risk assessment is the process in which information is analyzed to determine if an environmental hazard might cause harm to exposed persons and ecosystems.* The process consists of four steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization. The original description for each step as defined in the Red Book (NRC 1983) is as follows: "*hazard identification is the determination of whether a particular chemical is or is not causally linked to particular health effects; dose response is the determination of the relation between the magnitude of exposure and the probability of occurrence of health effects in question; exposure assessment is the determination of the extent of human exposure before or after application of regulatory controls; and risk characterization is the description of the nature and often the magnitude of human risk including uncertainty*." The 1983 definitions might appear outdated as current risk assessment documents have modified and improved such definitions, but there is value for a beginner in the field to understand and appreciate the original definitions from the historical point of view. The EPA historical perspective of risk assessment is complicated, but for the purpose of this introductory book a short discussion will suffice. For a more comprehensive perspective, the reader is encouraged to read the references.

The first EPA risk assessment document was completed in December of 1975. This document targeted quantitative risk assessment for community exposure to vinyl chloride (EPA Staff Paper 2004). In 1976, the EPA produced another significant document related to risk assessment entitled "*Interim Procedures and Guidelines for Health Risk and Economic Impact Assessment of Suspected Carcinogens*." Later in 1976, as a result of these two documents, the EPA recognized that rigorous

assessment of health risk and economical impact would be undertaken as part of the EPA regulatory processes. In 1980, the EPA listed 64 water contaminants and this was the first application of quantitative procedures used on risk assessment. The National Academy of Science (NAS) published Risk Assessment in the Federal Government: Managing the Process "Red Book" in 1983, which detailed the requirements and procedures for risk assessment. In 1984, the process improved with increased transparency and required assessment of the strengths and weaknesses as well as recommending the use of quantitative alternatives to the EPA (qualitative) approach to uncertainty analysis. In 1986, the EPA began publishing guidelines to conduct risk assessment. In 1992, the field moved forward again when the EPA adapted human health risk assessment (HHRA) for ecological risk assessment (ERA). This was considered a major advance as previously risk assessment had focused solely on human health. The EPA drafted a process to protect the environment, population and ecosystems. Also in 1992, the EPA published guidelines for estimating exposures. In 1994, NAS published the "*Science and Judgment in Risk Assessment*" report. The following year, 1995, was another key year for the risk assessment process. The EPA updated a risk characterization policy that recognized the necessity of transparency and clarity on the risk assessment process. This policy promoted that all risk assessment developed at the agency include a risk characterization step to improve risk assessment. In 1997, the Congressional Presidential Commission on Risk Assessment and Risk Management (CRARM) was created to investigate appropriate uses of risk assessment and management in governmental regulatory programs. CRARM published two reports: Better Understanding and Quantification of Risk, and Strategies to Reduce Human and Ecological Risk. In 2000, the EPA published the characterization handbook to implement the 1995 risk characterization policy of transparency, clarity, consistency, and reasonableness (TCCR). More recently in 2009, the National Research Council (NRC) released "*Science and Decision: Advancing Risk Assessment*" with a more comprehensive framework that included stakeholders (individuals with personal interest or affected due to risk assessment outcome) involvement, expanded the risk assessment process to three phases and reinforced the importance of uncertainties, variabilities, and improving the utility of risk assessment.

The *goal* of the risk assessment process is to protect life (human and ecological). The primary *purpose* of risk assessment is to inform the risk manager's decision-making process. The current state-of-the-art risk assessment frameworks provide both a better understanding and systematic processes to address risk beyond a yes/no type of answer. Table 1.1 shows the major advancements over time.

Many scientists and policy experts developed the risk assessment process. However, as a scientific endeavor, the practice and implementation of risk assessment by the scientific and regulatory community has spanned several decades since its inception and criticism continues to this day. Criticism is primarily due to the fact that the risk assessment process is not entirely a scientific endeavor and requires the use of expert (value) judgment in many cases. Without question, the risk assessment process continues to evolve and improve on the basis of scientific feedback.

From the public point of view, the philosophical importance of why the EPA should even consider conducting risk assessment is described by the EPA's mis-

Table 1.1 Selected milestones improving the risk assessment process since the publication of the NRC Red Book in 1983. (Information adapted from NRC Red Book)

Year	Milestone
1983	NRC's Risk assessment in the Federal Government Report (the Red Book)
1986	EPA's expanded 1976 guidelines for carcinogen Risk assessment
1991	EPA's expanded 1986 guidelines for developmental toxicity
1992	EPA framework for Ecological risk assessment
1993	NCR's Pesticides in the diets of infants and children
1994	NRC's Science and Judgment in risk assessment
1996	EPA's guidelines for reproductive toxicity
1997	Exposure factors handbook; Presidential commission on risk assessment and risk management report
1998	EPA's guidelines for neurotoxicity and/ ecological risk assessment
2000	EPA's risk characterization handbook
2003	EPA's framework for cumulative risk assessment
2004	EPA's risk assessment principles and practices staff paper
2005	EPA's expanded 1986 guidelines for carcinogen risk assessment
2006	EPA's framework for assessing health risk of environmental exposure to children.
2009	NRC's Science and Decisions: Advancing Risk Assessment (the Silver Book)
2014	NRC Review of EPA's IRIS Process

sion statement (EPA Staff Paper 2004)—*To protect human health and safeguard the natural environment.* By performing risk assessment, the EPA helps government agencies to make informed decisions in setting environmental standards and regulation. The risk assessment process helps risk managers to reach informed decisions even when limited data is available, but an action is necessary. Sir Bradford Hill expressing the need for action even when not all pieces of the puzzle are known, states: "*All scientific work is incomplete (whether is observational or experimental). All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us a freedom to ignore the knowledge we already have, or to postpone the action that it appears to demand at a given time,*" (Hill 1965).

In their own words EPA conducts risk assessment to provide the best possible scientific characterization of risk based on a rigorous analysis of the available information and knowledge. The assessment informs the risk manager about the scientific implications of the risk in question. Nevertheless, a unique caveat is that the risk assessment process uses existing scientific information to organize, analyze, synthesize and reach a conclusion and make recommendations of the possibilities

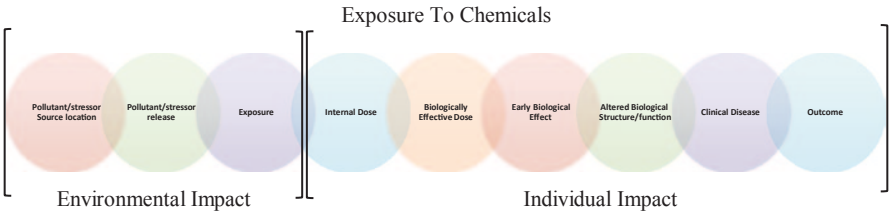

Fig. 1.1 Exposure to chemicals. (Adapted from Schulte 1989)

of risk, but does not generate new knowledge of the scientific subject matter or the issue in question.

Humans have been exposed to potentially harmful chemicals since the beginning of time. However, the birth of the industrial revolution changed the rate, quantity and frequency of chemical exposures increasing the chemicals (natural or man made) humans will encounter in the course of a lifetime. Although the number of chemical products increases, often the knowledge of the adverse effects of new chemicals products is not well understood. As societal needs increase the demand for production, quantity and availability, the chance of human and environmental exposure also increases. Human exposure may come from several sources such as air, water, soil, food, job occupation etc. An important concept to understand is that exposure does not translate equally to human internal dose. The chemical exposure in humans needs to move across barriers and also faces variability. These barriers and variabilities are present from human exposure directly to the chemical or stressor or indirectly due to environmental exposures. Figure 1.1 illustrates the complexity of this process and how a stressor may possess a range of risk that may include both ecological and/or human health impacts. The chemical agent confronts many barriers and sources of variability until the final outcome is observed. As science becomes better at understanding and measuring chemicals and stressor exposure, the information used for developing risk assessment becomes more precise resulting in improved decision making to protect public health.

Risk Assessment in the Federal Government: Managing the Process (commonly called "the Red Book") offers the first seminal treatment of the risk assessment process. The emphasis of the Red Book is human health risk assessment. The risk assessment process differs between what is known as human health risk assessment (HHRA) and ecological risk assessment (ERA). The reason for this separation is that HHRA is primarily concerned with individual human exposure to single chemicals. ERA is primarily concerned with adverse ecological effects as a result of exposure to one or more chemicals or stressors (US EPA 1992). Furthermore, the EPA also uses the cumulative risk assessment (CRA) framework to help with understanding situations from aggregate exposure (multiple route of exposure) to multiple stressors (biological, chemical and physical) (US EPA 2007). ERA was introduced in 1992, and uses the basic principles layout in the HHRA process with some modifications. This book (Toxicological Risk Assessment for Beginners) focuses on the HHRA framework due to its straightforward approach. Therefore, if

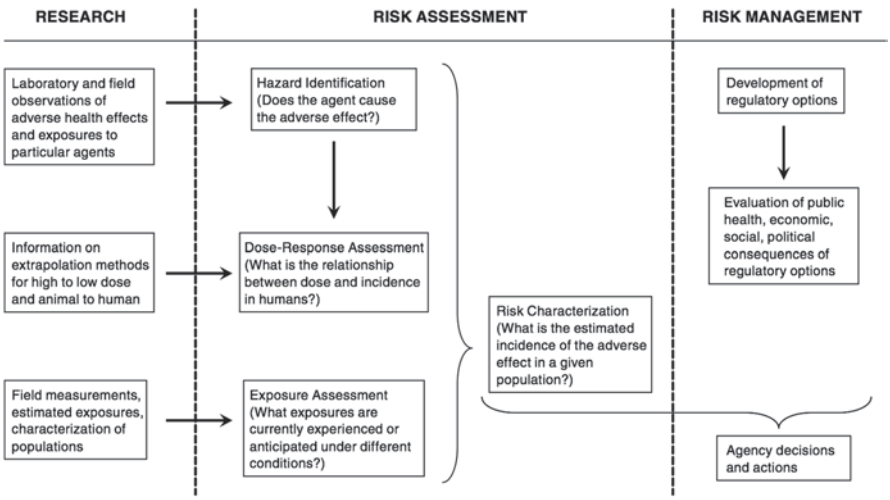

Fig. 1.2 NAS original risk assessment framework as appeared in the 1983 Red Book. (Risk Assessment in the Federal Government: Managing the Process)

the reader acquires a good grasp of HHRA process, s/he will be able to understand other frameworks. Although the processes are not entirely similar, the endeavor should not be as difficult to compare as starting from scratch.

For illustration purposes Figs. 1.2, 1.3, 1.4 and 1.5 show HHRA, ERA, CRA respectively, and one integrated framework that combine HHRA & ERA together. The purpose of showing all these frameworks together is (1) to provide a big picture overview; (2) to create an awareness of the several frameworks in existence and use; and (3) to show the flexibility and adaptability of these frameworks depending on the need or problem to solve. Even though the book's focus is the HHRA framework, a basic awareness of other frameworks is not out of scope for this introductory chapter. The HHRA framework was originally designed for U.S. government agencies, but the framework is now used outside the government in places such as the European Union, the World Health Organization, Latin America, and others. As Fig. 1.2 illustrates, the NAS framework shows a scientific research component that generates the information to feed the process. It also provides a risk assessment process to analyze the information and a risk management component to value the options and inform decision-making for protecting the population. The risk assessment process is at the core of this framework.

The ERA incorporates the planning and problem formulation steps to help formulate and select the appropriate questions, proper assessment and endpoints to be measured. Because ecosystems are composed of many populations and not single individuals, this framework very closely integrates the characterization of exposure and characterization of ecological effects. Landis (2004) broadly describes an ecological effect as *any impact upon a level of ecosystem organization*. ERA brings together the exposure assessment and dose-response (effects) of the original HHRA

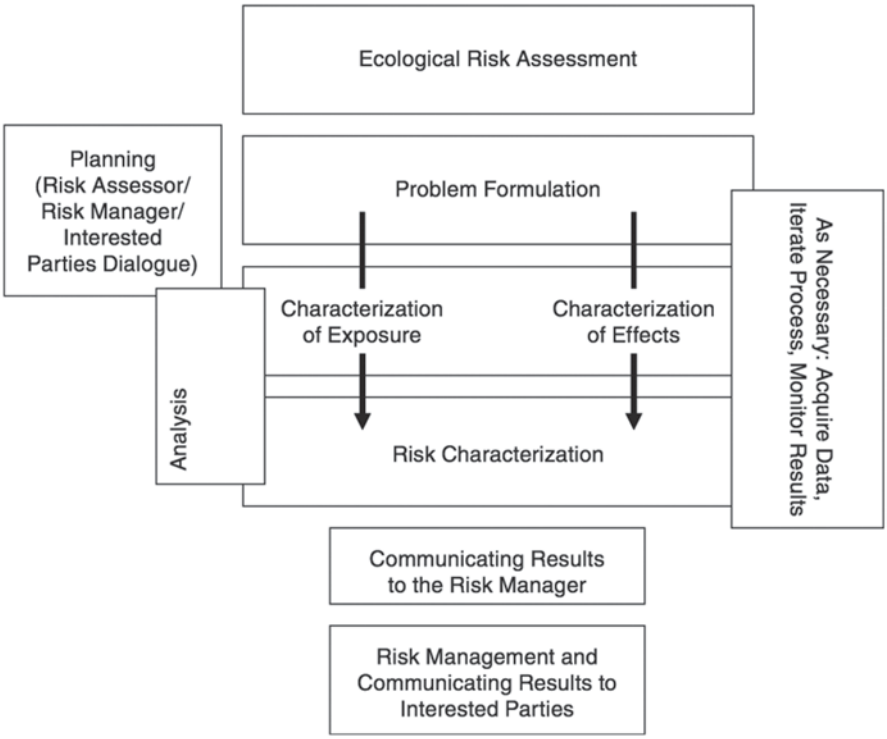

Fig. 1.3 EPA framework for ERA. (Source: NRC 2009)

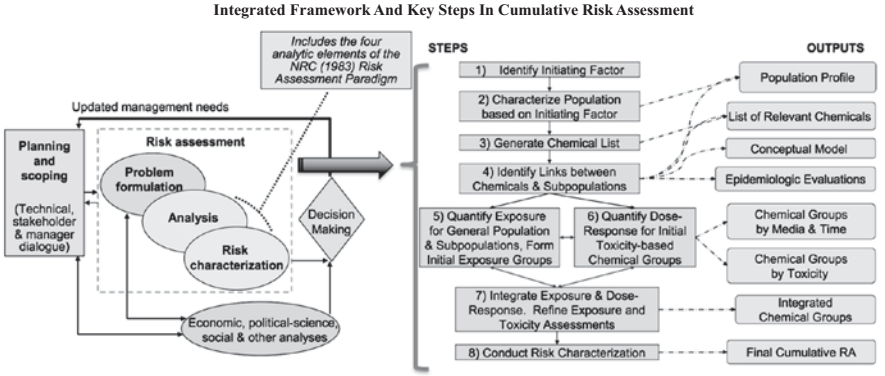

Fig. 1.4 Modified from concepts, methods and data sources for cumulative health risk assessment of multiples chemicals, exposures and effects: a resource document. (EPA 2007)

framework and makes appropriate modifications to adjust for the ecological systems. This combination results in a stressor-response profile that in many ways resembles the dose-response in HHRA, but expands to community and ecosystem

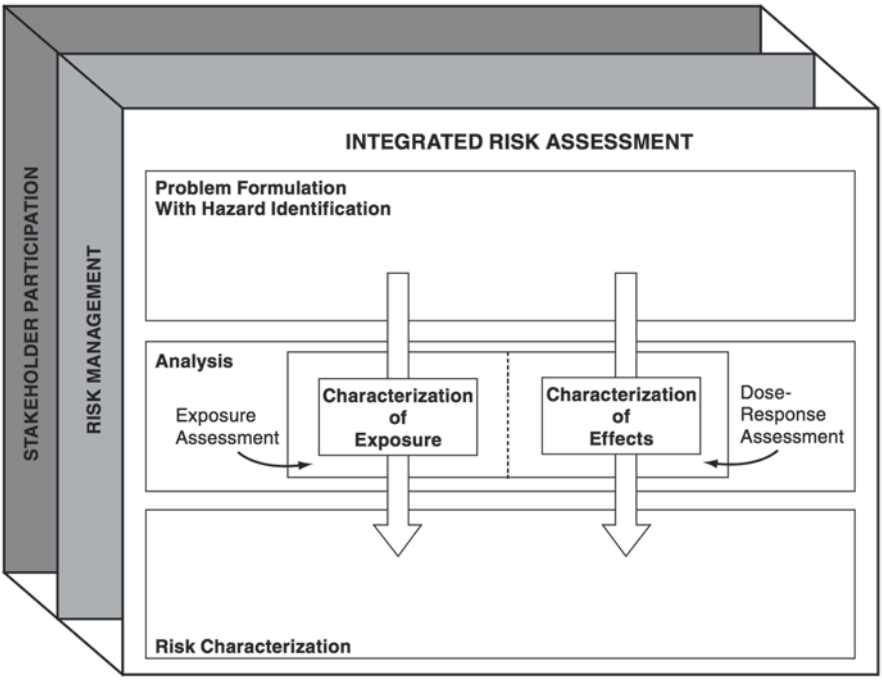

Fig. 1.5 Integrated human health and ecological risk assessment framework. (Suter 2005)

levels (Landis 2004). The ERA model, as depicted in Fig. 1.3, asks the question is this chemical or stressor bad for the ecosystem? The HHRA asks a similar question for human exposure.

The EPA acknowledges that in real life humans are not exposed to a single chemical or stressor and exposure to multiple agents or stressors is closer to reality. The cumulative risk assessment framework intent is to address this concern by moving from a single exposure to the combined aggregate of exposures by analyzing, characterizing and quantifying the possible risk to humans or environments. The cumulative framework is not only from multiple chemical exposures but also includes non-chemicals stressors (i.e. chemical X + radiation + chemical Y + nutrition). The EPA cumulative health risk assessment document (EPA 2007) specified the three initiating factors that may be used to conduct a cumulative risk assessment as (1) multiple pollutant sources or releases, (2) elevated concentrations from environmental monitoring or biomonitoring of chemicals, and (3) increased population illness in a community. But this is not to be confused with the EPA framework to combine only chemical mixtures (chemical x + chemical y + chemical z). The chemical mixture chapter in this book will address appropriate frameworks and the current state-of-the-art information on combined exposures. Figure 1.4 shows a general framework with specific key steps used in cumulative risk assessment (EPA 2007).

The HHRA, as shown previously, has been adapted for ecological and cumulative risk assessment by the EPA. Outside the U.S., these frameworks are also being utilized for risk assessment such as the model from the World Health Organization (WHO) that integrates HHRA and ERA in a single framework. The necessity for these integrations is explained by the WHO (2001) as *For practical reasons, the methodologies for human health and ecological risk assessment developed independently. However, with increased recognition of the need to more effectively protect both humans and the environment, it is time to consider a move to a more integrated, holistic approach to risk assessment*. The WHO model provides five major benefits for this integration: (1) coherent integrated results that provide basis for action to support decision-making; (2) interdependence of human and ecological risk that might gather important modes of action and interactions between effects on the humans and on the environment; (3) the use of sentinel non-human organisms to identify potential environmental sources of humans hazards; (4) sharing information and techniques to improve the scientific quality assessment; and (5) an increase in efficiency through integration.

Silver Book Framework

The National Research Council published *Science and Decision: Advancing Risk Assessment* in 2009 (NRC 2009). Colloquially referred to as "The Silver Book," this is the latest, significant update to the HHRA framework. The silver book incorporates three phases for a new integrated framework to improve the utility of risk assessment. Additionally, in 2013 the NRC published the report: Environmental Decision in the Face of Uncertainty. This report expands upon the 2009 framework to reflect the importance of handling uncertainty in health, technological and economical factors (NRC 2013). The three phases below are presented accordingly to the NRC 2009 framework (NRC 2009).

Phase I: Problem Formulation and Scoping

This phase provides the beginning framework to help risk assessors set the stage with pertinent questions for dissecting the problem at hand and identifying proper boundaries. Phase I questions include (NRC 2009) (1) what problems are associated with existing environmental conditions; (2) if existing conditions appear to pose a threat to human or environmental health, what options exist for altering those conditions; and (3) under the given decision context, what risk and other technical assessment are necessary to evaluate the possible risk management options? Furthermore, when initiating a risk assessment process, the purpose of bringing together decision makers, stakeholders and risk assessors early in the process is to increase communication, understanding and clarity of the common goal they seek to pursue. This

is important because the very best and most competent risk assessor is not shielded from the effects of decision-making elements in our society. To increase project success and avoid inefficient use of energy and effort, a better approach is early incorporation of all stakeholders for effective project management.

Phase II: Planning and Conduct of Risk Assessment

Three stages are the main core of this phase (NRC 2009). Stage 1 is planning; stage 2 is the risk assessment process (hazard identification, dose-response, exposure assessment, risk characterization), and stage 3 is confirmation of utility. Planning (stage 1) focuses on identifying the attributes of assessment necessary to characterize risk of existing conditions. This stage determines the goals, specific risk scenarios, required levels of risk quantification including variability and uncertainty, and identifies critical data gaps and risk after application of risk management options. This is based upon the given decision-context or framework created in problem formulation and scoping.

The risk assessment process is found in stage 2. The reader might benefit from visualizing the risk assessment process in this framework as a gear that was inserted into a larger and more complex machinery containing other gears. Figure 1.6 illustrates the interrelation of the three phases. Thus, in the new integrated framework the risk assessment process is not isolated, but at the core of phase 2 (stage 2). As previously mentioned, stage 2 holds the basic steps of hazard identification, dose-response, exposure assessment and risk characterization.

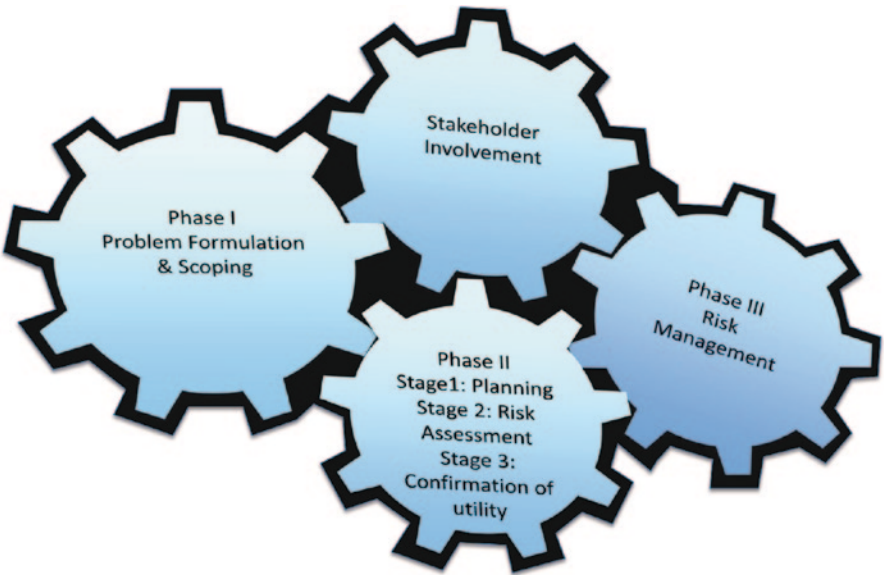

Fig. 1.6 Representation of Silver Book integrated framework

The stage 3 is confirmation utility (NRC 2009). Stage 3 verifies that the information collected during the assessment has the attributes previously identified during planning (is there sufficient information to create and discriminate among multiple risk management options, and has the assessment has been satisfactorily peer reviewed?) When the answer is "no" to the questions posted in the confirmation and utility, the process is to step back to planning (stage 1). Otherwise, if successful, the process moves to phase III.

Phase III: Risk Management

At this point the risk manager or decision maker (not the risk assessor) is managing the implications of the relative health or environmental benefits of the proposed actions. The process may be presented in two steps (1) analysis of risk management options and (2) selected decision, implementation and communication. The main issues addressed during this phase are (a) outlining the proposed action effect against other decision-making factors such as technologies or cost; (b) evaluating and justifying the decision against the benefits, cost and uncertainties of said decision; and (3) selecting the best approach for communicating the decision (NRC 2009). The risk manager may include an assessment of the effectiveness of the decision reached and, if necessary, how that assessment will be accomplished. The risk management process is out of the scope for this book and will not be covered any further.

Interactions

The three phases also provide information to stakeholders (internal or external) to keep them involved at all stages of the framework. However, this is not intended to compromise the technical assessment of risk developed by the risk assessor in the phase 2/stage 2. The risk assessment process should be viewed as a scientific endeavor (although not entirely true due to expert judgment utilization) and carried out under proper standards and guidelines.

These frameworks offer a flexible approach to organize and analyze complex scientific data that was originally collected for research purposes (not designed to serve the specific purpose of risk assessment) but is now used to design risk assessment and answer questions of risk. Frameworks are not fixed models as the reader may appreciate from the evolution of the original HHRA, modified for ERA, adapted for CRA, and integration as presented by World Health Organization. These are by no means the only frameworks available to develop risk assessment; modifications include microbial risk assessment guidelines, risk assessment guidance for superfund (RAGS), guidelines for neurotoxicity risk assessment, etc. The intention is not to present every single framework and guidelines, but rather provide a big picture perspective so that the reader creates a mental map while reading through this book or as s/he reads information related to risk assessment from other references

and sources. The main messages are: (a) the frameworks are not static, but rather a flexible matrix; and (b) the original HHRA framework from 1983 was developed by NAS for the purpose to guide decision making in the USA government and is now adapted in many non-government sectors and non-USA locations.

Summary

Understanding a complex issue such as risk requires the process to be broken down into several steps. These include: Hazard Identification, Dose Response, Exposure Assessment (when applicable) and Risk Characterization. Risk assessment is the process where the basic questions of "How Bad?" and "How Much?" can be combined into a controlled, measurable scenario, allowing risk managers and stakeholders to make informed decisions. Risk assessment is relatively new as a professional practice. The framework presented by the National Academy of Sciences has evolved over time, as technology and scientific understanding has evolved. Integrated and evolving approaches to risk assessment have been discussed to make the reader aware of the ever-changing nature and practice of risk assessment.

Both this chapter and the full content of this book are intended offer the reader a basic understanding and solid building blocks of details sufficient for developing a basic working knowledge of the risk assessment subject. The reader is encouraged to continue advancing his/her skill beyond the information contained in this book. Chapter 11 discusses resources and skills and provides information on acquiring an electronic copy of the silver book and other important skills for continuing professional development in the risk assessment field.

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Chapter 2
Hazard Identification

Tiffany Bredfeldt and Daniel E. Arrieta

Abstract Hazard identification is considered an early step in the risk assessment process. The primary goal of this step is to determine whether exposure to a chemical is likely to cause a specific adverse health effect in humans. The process of hazard identification consists of collecting, evaluating and integrating various sources of data to produce a scientifically-defensible conclusion regarding stressor-induced causation of adverse health effects. The product of data integration is a weight of evidence narrative that characterizes the conditions under which exposure to a chemical is likely to harm human health. This chapter provides a basic introduction to the concept of hazard identification, information critical to this step in risk assessment, and evolving trends in hazard identification.

Keywords Hazard identification · Weight-of-Evidence · Bradford Hill criteria · Database evaluation · Mode of action · Critical effect · Molecular initiating event · Adverse outcome pathway · read-across

Student Learning Objectives

The goals of this chapter are:

- To learn the process that goes into making a judgment regarding the effect(s) caused by an agent of concern

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- To understand how to evaluate the available database to determine if there is evidence of causation
- To define mode of action and critical effect
- To discuss evolving trends in hazard identification

Hazard Identification Definition

Historically, hazard identification has been considered as the first step of a risk assessment (OSTP 1985; NRC 1983). Later permutations of the early steps of the risk assessment process include, in addition to hazard identification, steps of planning and scoping and problem formulation, which are discussed elsewhere in this book (USEPA 1992, 1998, 2003, 2004; NRC 2009).

The goal of the hazard identification step is to make a scientifically defensible judgment about whether exposure of the human population to a given stressor, typically but not limited to a chemical of concern, causes a specific adverse health effect. This process requires a detailed evaluation of available data which is then used to generate a weight-of-evidence analysis that supports or opposes the hypothesis that a stressor is causal of a given adverse health effect in humans (OSTP 1985; NRC 1983). It is also the intention of hazard identification to classify the types of adverse health effects a given stressor may cause. The broad classification(s) assigned to such stressors could include: carcinogen, developmental toxicant, reproductive toxicant, immunotoxicant, neurotoxicant, hepatotoxicant, nephrotoxicant, pulmonary toxicant, cardiotoxicant, dermal toxicant, ocular toxicant, et cetera. A chemical stressor could also be classified as toxic to numerous organ systems, which may be targeted concomitantly upon exposure, at certain concentrations/doses[1], or depend on the route of exposure itself (e.g., inhalation, oral, dermal, ocular) (USEPA 1996, 1998, 2005; NRC 2009).

The Database Evaluation

A key aspect in determining the potential hazard associated with a chemical agent, which will be the stressor discussed from this point forward, is identifying what information is available upon which to draw a conclusion. Data used in hazard identification varies. However, studies conducted in humans or on exposed human populations offer the strongest support that exposure to a given chemical stressor causes an observed adverse health effect. While human studies may offer the most compelling evidence for hazard identification, they are often few in number or weakly informative as they may represent the simple observation that a chemical is

[1] The word concentration depicts exposure via inhalation whereas dose indicates exposure by oral route.

associated with an adverse health effect and lack mode-of action data. Thus, many hazard identifications are conducted using animal data where inherent uncertainties exist regarding the relevance of such data to human health. Recent efforts, particularly those of the International Life Sciences Institute Risk Sciences Institute and the International Programme on Chemical Safety, have generated a variety of frameworks and guidance documents to aid in determination of whether data collected from an animal study is indeed relevant to human health (Boobis et al. 2006, 2008; Meek et al. 2003; Meek 2008; Seed et al. 2005; Sonich-Mullin et al. 2001).

Risk assessors rely on a database of information that has been developed during the assessment of chemical toxicity using laboratory animals or epidemiological studies that consider adverse effects associated with exposure. This data becomes integral to establishing whether or not a chemical agent can cause an adverse effect in humans. As one moves along the process of making this determination, it is important to correctly identify the specific chemical of interest for a risk assessment (WHO 2012). In doing so, one can proceed with identifying what information is available to evaluate the intrinsic hazard associated with the chemical agent in question.

There are numerous public databases that can be queried for information regarding chemical-specific toxicity. Examples include government databases (i.e. TOXNET, IRIS, and NTP), peer-reviewed journals, and published books (U. S. EPA 2009). Typically, information is publically available and the content can be easily retrieved or requested from an academic institute. In addition to the sources identified above, there are also proprietary study reports developed by chemical manufacturers. These reports are not always accessible. However, summaries of these studies are available on the Organisation for Economic Co-operation and Development (OECD) eChemPortal website for chemicals sponsored under the OECD SIDS HPV Programme or USEPA High Production Volume Chemicals Program (OECD 2008; USEPA 1990). These reports come in the form of a well-written robust study summary. More recently, the European Chemicals Agency (ECHA) made available on their website physical-chemical, environmental fate, and toxicological data submitted during the process of chemical registration. Unfortunately, the amount of data available varies by chemical. However, the aforementioned databases can be utilized to survey the types of studies available to investigate the chemical of concern. As one moves along the process of identifying and gathering information required for the risk assessment, the intent is to identify studies that are deemed scientifically-defensible meaning they have undergone peer review or conducted according to standardized protocols approved by various regulatory bodies such as the United States Environmental Protection Agency or Food and Drug Administration.

Sources of information available on chemical agents include human clinical or epidemiological studies, *in vivo* or *in vitro* laboratory animal studies, mechanistic or kinetic studies, or computational toxicology (i.e., quantitative structure activity relationship, systems biology) (USEPA 2012). As a general rule, the use of human data is given higher importance than animal studies and most often preferred by a risk assessor (ECETOC 2009). However, before this information can be used in a risk assessment, it must undergo a rigorous review to determine the applicability

to use the data. When considering human data one must assess the appropriateness of the study design, determine the level of exposure information available and the health outcome. The risk assessor needs to clearly identify the appropriateness of the study design in relation to the types of groups used for comparison, time between exposures, adjust for confounding variables when necessary and determine the appropriate use of statistical analysis used to aid in the interpretation of the data (ECETOC 2009). Although there are no standardized protocols available to aid in assessing the integrity of the study design and interpretation for epidemiological studies, recent efforts to develop a systematic approach to yield greater transparency and reproducibility reviewing these types of studies has been proposed (Money 2013). More importantly, not all data obtained from epidemiological or case studies will address the descriptions provided above, therefore it is important to identify additional studies demonstrating some level of causal association related to a particular health outcome to provide the risk assessor with a higher level of confidence that the classification(s) assigned to a chemical of concern are accurate. More detail on assessing epidemiological studies is provided in a later chapter.

Risk assessors, as mentioned earlier, use animal based toxicological studies when human studies (e.g., case or epidemiological studies) are limited. Toxicological studies developed using standard methodologies approved by government agencies such as the United States Environmental Protection Agency (USEPA), Food and Drug Administration (FDA), or European Chemicals Agency (ECHA) are conducted under Good Laboratory Practices (GLP) and deemed of higher quality to be used in a risk assessment. Some considerations are necessary when evaluating these types of studies for use in hazard identification, for instance: validity of the methodology, reproducibility, study reliability, and appropriateness or usefulness of the study for the risk assessment (Bevan and Strother 2012). As for data developed not using standardized methodologies, this will require more effort to become familiar with the methodologies and relevance of the findings when evaluating the quality of this type of information.

One popular approach for evaluating the reliability of a study is the use of the Klimisch Code (also referred to Klimisch Scores). Klimisch et al. (1997) developed criteria to evaluate toxicology and ecotoxicology data. Three components for evaluating a study being considered for use in hazard identification and subsequent risk assessment were defined as: reliability, relevance and adequacy. Reliability of a study report or publication establishes whether or not the information was collected using standardized methodologies with sufficient details of the experimental design that are described in such a way as to provide evidence of the findings in relation to the clarity and plausibility. The extent to which data are appropriate for use in hazard identification or risk assessments relates to the relevance. Adequacy is defined as making a determination on the usefulness of the data to be considered in a risk assessment.

The Klimisch Codes have become adopted by programs such as the US High Production Volume Program, OECD-SIDS program, and European Union REACH legislation (USEPA 2005; OECD 2008; EU 2006). Another approach that has recently garnered some attention is the use of the ToxRTool (Toxicological data Reliability Assessment Tool). Schneider et al. (2009) developed the tool with the intent

on providing a transparent approach for assessing the reliability of toxicological data. There are two parts of the tool to assess both *in vivo* and *in vitro* studies and key parameters established to aid in the transparency and to harmonize approaches of reliability assessment. In addition, this approach is also useful for identifying the potential sources of variability associated with the evaluation of toxicological studies by various individuals.

Provided that epidemiological and animal studies will be the predominance of information available on a chemical agent at this time, there has been a concerted effort to identify ways to merge this information to aid risk assessments. Adami et al. (2011) recently proposed utilizing the Epid-Tox Framework to describe the strength of association between a toxicological effect and epidemiological information in a scalable form to establish a causal relationship between a chemical agent and an effect. The framework proposes using the following steps:

a. collect all relevant epidemiological and toxicological studies
b. assess the quality of each study and assign it to a quality category
c. evaluate the weight of evidence of the epidemiological and toxicological studies
d. assign a scalable conclusion to the biological plausibility and epidemiological evidence
e. determine the placement in a causal relationship grid

One example of the utilization of the Epid-Tox Framework described by the authors related to the adulteration of milk with melamine reported in China (Adami et al. 2011). It has been generally recognized that bladder and kidney toxicity seen in animal studies was considered relevant to humans, but primarily at very high concentrations. However, crystals found in children with melamine exposure in urinary bladder and confirmed deaths provided some corroborating evidence of a mode-of-action (MOA) seen in animal studies at high concentrations (WHO 2009). This type of information provided further support for the biological plausibility regarding human exposure to melamine and concerns with bladder and kidney toxicity. Simpkins et al. (2011) also reported the applicability of this framework by investigating the causal relationship between atrazine exposure and breast cancer in women. They concluded the absence of epidemiological evidence and lack of a plausible MOA associated with mammary tumorigenesis in female Sprague Dawley rats did not support public concerns related to the carcinogenicity of atrazine and was in-line with the previous schemes for the classification of carcinogenic potential of atrazine in humans reviewed by others (USEPA 2003, 2006) and IARC (1999).

In a similar direction and effort, Lavelle et al. (2012) have also proposed a framework aimed at systematically integrating human and animal data with the intent of creating consistency and transparency in the process for the purposes of evaluating and classifying chemical agents. Please refer to Fig. 2.1 for an illustrative example regarding the application of this framework to be used for a chemical risk assessment. As seen from this example, the integration of data from available studies enables a conclusion to be drawn regarding the causal relationship between a chemical agent and an adverse effect.

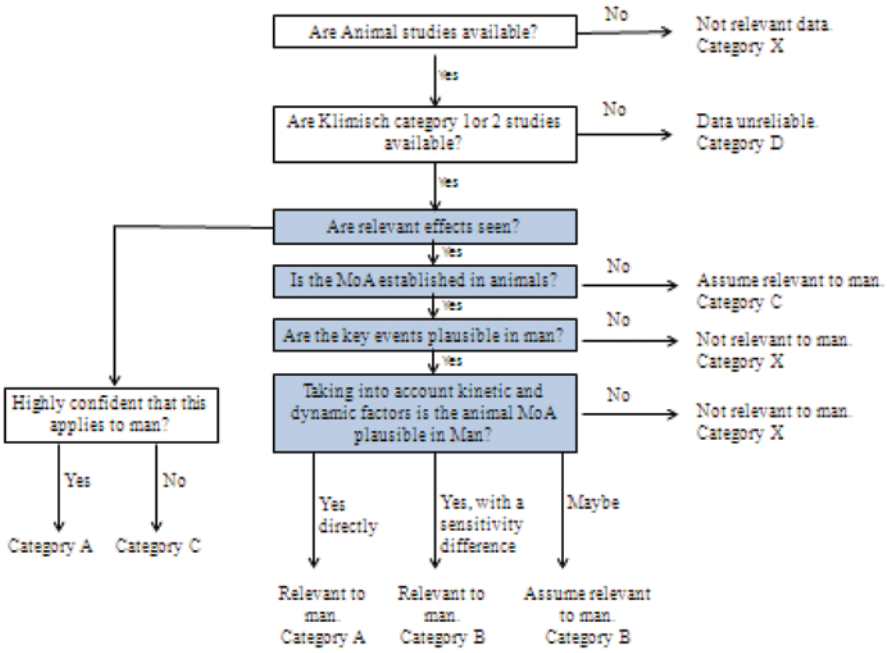

Fig. 2.1 Illustrates an approach to categorize animal data to determine the relevance in human risk assessment. (Reprinted from Regul. Toxicol. Pharmacol, 62/2, Lavelle et al. 2012, with permission from Elsevier)

Once all data have been identified for conducting a risk assessment, the next step in the process is to determine what critical effect is associated with the chemical of concern. Although in principle this may seem a fairly straight forward process, in actuality there are a number of factors described below that need to be considered and understood before drawing a conclusion. For example, expert judgment in evaluating the quality of studies and suitability for hazard identification to be used in risk assessments are important factors to consider. In addition, identifying an effect seen in animal studies between controls versus treatment groups, establishing if there is clear evidence of a dose response observed with the treatment groups, assessing whether the effect is adverse, and the biological significance of the reported effect are all important for the risk assessor to take into consideration (Dorato and Engelhardt 2005; Lewis et al. 2002). To gain a better appreciation for these types of challenges, the reader is encouraged to follow up with the work submitted by Lewis et al. 2002. The authors provide a comprehensive approach by outlining criteria for establishing whether the observed effect is treatment-related and whether the effect seen in animal studies is adverse.

For toxicological studies, dose-related responses identified as statistically different from the control group are evaluated as potentially adverse. The portion of the dose response where control and exposed organisms are not different is commonly referred to as a No-Observed-Adverse-Effect (NOAEL). It is an important determinant in establishing whether there is a concern related to an observed target organ effect (USEPA 2012). There have been numerous definitions provided by

various regulatory bodies or organizations describing the NOAEL, but generally speaking it is the highest concentration of a chemical of concern not shown to cause an adverse effect such as: alteration in morphology, functional capacity, growth, or developmental life span determined by experimental design or observation (WHO and WHO 1996). As the risk assessor, one needs to have a clear understanding of what constitutes an adverse effect so that a determination can be made about the relevance of that observed effect to human health.

One of the most frequent toxicological effects reported in animal studies and relevance to humans is described as α_{2u}-gobulin and nephropathy seen in male rats (USEPA 1991; Swenberg 1993). This commonly reported effect observed in male rats in association with renal carcinogenesis has little or no human relevance. Another commonly reported adverse effect seen in toxicological studies related to exposure to chemicals that induce hepatic enzymes is liver hypertrophy. Chemical-induced hepatic (liver) hypertrophy is well-documented in rodent studies. However, the significance of this observed effect has been questioned. Liver hypertrophy as defined by toxicologists can have various meanings such as; increase in liver weight (liver hypertrophy), increase in average size of hepatocytes (hepatocellular hypertrophy), and hepatic enzyme induction (work hypertrophy) (Hall et al. 2012).

Recently, the European Society of Toxicologic Pathology (ETSP) convened an expert opinion group to discuss the significance of hepatocellular hypertrophy in rodents to establish whether this was an adaptive or adverse response (Hall et al. 2012). The opinion reached by the expert group was that hepatomegaly (enlarged liver) in the absence of histopathological or clinical pathology changes associated with liver toxicity was considered to be an adaptive response and should be reached using a weight of evidence approach. The expert group also stated that hepatocellular hypertrophy associated with the increase in liver metabolizing enzymes can be considered fully reversible and not expected to compromise the viability or functional integrity of the organism. The examples provided above emphasize the importance for identifying the mode-of-action (MOA) of a chemical stressor to characterize what adverse outcomes are associated with a molecular initiating event (MIE), and these concepts will be addressed later in this chapter.

Mode of Action Evaluation and Identification of Critical Effect

Chemical stressors may cause a plethora of responses in exposed organisms. The range of effects is often highly variable and driven by the manner in which exposure occurred, the duration of exposure, the dose, inter-organismal variability, and concomitant exposures. Often, one of the most dominant determinants driving the outcome of exposure is the dose to which the organism is exposed. Dose in this context not only refers to the concentration of chemical measured in a given exposure media such as air, soil, or water, but it also refers to the dose at a given target tissue inducing an adverse effect. It is important to consider chemical's characteristics (physical and chemical properties), which may affect its ability to be absorbed

into the body. Furthermore, it is important to consider what happens to the chemical upon absorption. The term toxicokinetics broadly refers to how a chemical is absorbed, what happens to it while it is in the body (i.e., distribution, metabolism), and ultimately how it is removed from the body via excretion. Data regarding these issues is more common to well characterized chemicals where a significant number of studies have been conducted to evaluate toxicokinetic properties. After absorption, the effects of exposure are often described as a series or continuum of effects that are manifest in dose- and duration of exposure-dependent manner. From this perspective, the response to a given exposure may escalate from mild physiological adaptations, to compensatory stress response, then progress to the induction of an apical effect, and finally to the manifestation of an adverse effect (Dourson et al. 2013). This process may be referred to collectively as toxicodynamics.

The sequence of molecular key events that occur prior to the manifestation of an adverse effect is called the chemical's mode-of-action (MOA). It is the identification of the apical effect that is relevant to human health that is crucial during hazard identification. The apical effect is the key event that happens immediately prior to the adverse effect, making it a molecular gate keeper of sorts. Thus, during the evaluation of chemical-specific toxicity data, priority should be given to data collected in humans. When quality data is not available in humans, animal studies may be a source of information. However, it is not the data collected in the most sensitive animal species that matters most. Rather, it is the health effects that are relevant to humans that should be considered to be of greatest concern. When the relevance of the adverse or apical effect to human health is unknown, data collected in the most sensitive animal species may be chosen as a means of conservative scientific judgment.

When considering whether or not an effect induced by chemical exposure is indeed adverse, it is important to define what an adverse effect is. There are several committees and organizations that have attempted to define adverse effect and a general consensus is that an adverse effect is:

- A change in morphology, histology, organ function, growth, reproduction, survival, longevity of a cell, development, of a tissue, organ system, or organism
- This change reduces the organism's ability to function, reduces the ability to respond to other stressors, increases susceptibility for disease or other dysfunction, and decreases the long-term chances of survival (Dorato and Engelhardt 2005; Keller et al. 2012; Lewis et al. 2002; NRC 2007; USEPA 1994).

An adverse effect is distinguishable from an adaptive response in that the change(s) constituting an adverse effect decreases survival of the organisms whereas an adaptive response enables the organism to respond to the stressor such that function is not reduced and survival chances are increased (Lewis et al. 2002; NRC 2007; Williams and Iatropoulos 2002).

Among the available sources of information regarding chemical-specific toxicity, it is important to identify possible adverse effect(s) caused by exposure and potential mechanisms driving those effects. Available data may be insufficient to identify the mechanism of action governing all observed adverse effects induced by chemical exposure. However, the generation and incorporation of more

high-throughput, molecular data is enabling a better characterization of the cellular pathways involved in both homeostatic or stress responses and the induction of dysfunction and damage. When highly detailed data are not available to fully characterize the chemical-specific mechanism(s) of action, the identification of chemical-specific MOA may be possible. MOA is distinguished from mechanism of action in that it is a less detailed description of the key molecular events that precede the manifestation of an adverse effect. The application of MOA is somewhat different than the mechanism of action. Where the mechanism of action is used to fully characterize the molecular events that occur to cause an adverse effect, the MOA utilizes a simplified scheme of events that are critical to the adverse effect (Fig. 2.2). A risk assessor benefits most from the MOA in a sense that it requires less data to generate and is part of the evaluation of dose-response that may lead to the genesis of toxicity factors to be utilized in regulation (Dellarco and Baetcke 2005).

Essential to hazard identification is a MOA evaluation (USEPA 2005). There are many MOAs that are the underpinnings of various adverse effects. This step is not only important for determining key events upon which to base a point of departure, but also critical in evaluating the human relevance of an observed MOA and subsequent adverse effect. Recent efforts have attempted to describe a framework to integrate MOA and human relevance together to allow for concomitant evaluation. The unifying element of this approach is to utilize Bradford Hill criteria for causation,

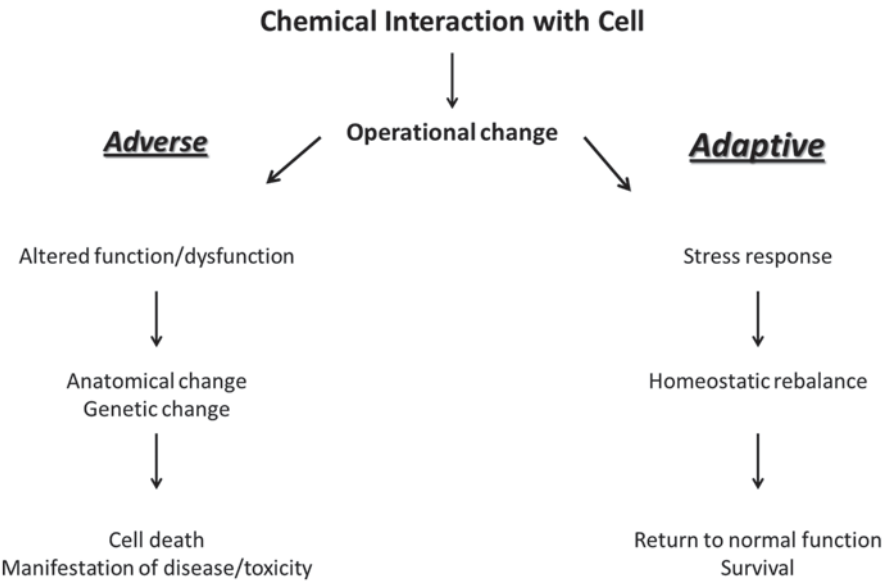

Fig. 2.2 Illustrates some of the key differences between potential cellular responses that may occur following chemical exposure. Responses in this figure may be characterized as either adaptive or adverse and both are part of the chemical-specific MOA. It is important to distinguish adaptive effects from adverse effects during risk assessment process as this distinction is the basis upon which the hazard identification and dose-response assessment are built

which are discussed later in this chapter, to determine whether the available data are adequate to develop a putative MOA and if that MOA is relevant to human health (Meek et al. 2003; WHO 2006; Sonich-Mullin et al. 2001). For data rich chemicals, additional details such as toxicokinetic and toxicodynamic data may be used to further inform the risk assessment using data rather than standard default approaches. Such information may also go beyond the scope of MOA evaluation and also aid in identification of subpopulations at greater risk (Meek 2008).

Evidence Based Evaluation of Available Database

The determination of causation is no simple thing. Epidemiological studies can be misleading by revealing an association between a chemical present in the environment and an adverse effect or disease when the observed effect is due to confounding or poor study design. Similarly, animal studies may indicate that chemical is, for example, a carcinogen when in fact the mechanism of carcinogenesis in the study animal species is not pertinent to human physiology. The hazard identification stage of a risk assessment is dominated by uncertainty regarding the cause and effect relationship that exists between exposure and adverse health effect. This uncertainty is centered around the concern of misclassifying a chemical agent or coming to an incorrect conclusion regarding causation.

To guide consistent decision making, guidelines are useful for facilitating the identification of causation. One such set of guidelines are called the Hill Criteria (Hill 1965):

- Strength: refers to how strongly the chemical of concern associates with the adverse effect or disease (*e.g.*, large relative risks or mortality ratios, high tumor incidence)
- Consistency: a chemical exposure that is observed to occur concurrent with the manifestation of a given disease or adverse effect in a number of independent studies is considered to be consistently associated
- Specificity: an adverse effect or disease is particularly associated with an exposure to a certain chemical and not with other types of exposure
- Temporality: the adverse effect of disease is observed after exposure to a chemical of concern
- Dose-Response: the magnitude and frequency of the adverse effect or disease is heightened when the exposure is increased
- Plausibility: indicates that a proposed mechanism for how a given stressor causes an observed adverse effect or disease is reasonable and biologically possible
- Coherence: based on what is known, the chemical of concern causes a given adverse effect or disease; no conflicting data
- Experimental Evidence: research in different models or types of experiments indicate that the chemical of concern can cause an observed adverse effect
- Analogy: various model systems or structurally related chemicals cause the same effect

When these above mentioned criteria are fulfilled for a chemical, the available body of data indicates that the chemical stressor found in association with a given adverse effect of disease state is due to it being the causal agent (Hill 1965). Importantly, not all Hill Criteria must be satisfied in a hazard identification (HI). In fact, it is often rare for the body of data characterizing a given chemical to actually satisfy all Hill Criteria. However, the more of these criteria are met the greater confidence an assessor may have regarding the ability of a chemical to cause an adverse effect (USEPA 1992).

It is difficult to prioritize which of the Hill Criteria are least important in the HI phase of a risk assessment; however, some criteria must be met for causation to be determined. During the HI phase, judgments regarding available data will vary among assessors and/or regulatory bodies. Despite these differences, it appears temporality, strength, dose-response, and consistency are the criteria considered necessary and integral to the demonstration of causation. In lay persons terms, meeting these criteria demonstrate that exposure to the chemical of concern occurs before the adverse effect (i.e., temporality and consistency). The more chemical exposure occurs the more severe the effect (i.e., a dose-response phenomenon is observed). In addition, the observation that chemical exposure has caused an adverse effect on more than one occasion (i.e., consistency), indicating that a hazard is not identified by a mere fluke study. Other Hill Criteria, in essence speak, to the quality and breadth of the available data regarding a chemical of concern. Thus, upon observation, they strengthen confidence regarding causation.

An excellent example of causation comes from cigarette smoking and lung cancer incidence and mortality. Many studies have demonstrated that smoking is associated with lung cancer, which occurs after a period of smoking (Blot et al. 1996; Surgeon General 1989; Shopland 1995; Wald 1996). This demonstrates a strong association and manifestation of the disease of cancer after exposure (*i.e.*, *temporality*). In addition, different analyses in human smokers and exposed animals indicate that smoking or exposure to chemicals in cigarettes is *consistently* associated with cancer. Based on what we know about the biological or physiological effects of chemicals found in cigarette smoke, which damage DNA and cause mutations, it is cigarette smoke a *plausible* carcinogen (Denissenko et al. 1996; Loft and Poulsen 1996; Pope et al. 2002). Available studies demonstrate that cigarette smoke is responsible for the observed lung cancer is *specific* due to the fact that no other confounding factors appear to be likely causal agents. There is also a positive *dose-response* relationship between the frequency and duration of smoking and the incidence of lung cancer. The *experimental* evidence across studies and in different animal models produces a body of data indicating that cigarette smoke and components therein are either tumor initiators or promoters. For example, several studies have demonstrated that when tobacco tar was painted on rodent skin it caused tumors. Likewise, complete smoke when administered via inhalation is tumorigenic. Taken together, these data indicate that cigarette smoke is a complete carcinogen (Engelbreth-Holm and Ahlmann 1957; Hoffmann et al. 1983 Orris et al. 1958; Wynder and Hoffman 1967). Based on the above discussion, it is apparent that cigarette

smoke meets all Hill criteria, which supports what is known about cigarette smoking: it causes cancer.

The Hill criteria could be used to evaluate any chemical of concern given there was sufficient data. However, not all chemicals of concern are as data rich as cigarette smoke. Thus, a greater degree of scientific judgment (i.e., value judgment) must go into the conclusion made by a risk assessor examining the available body of data. The process of assessing all available data in order to reach a consensus about what that body of data is in effect communicating to the evaluator is known as a weight of evidence (WOE) approach (Fig. 2.3).

The goal of a WOE approach is to produce a consensus statement about what is known about a given chemical so that available information may be used by risk

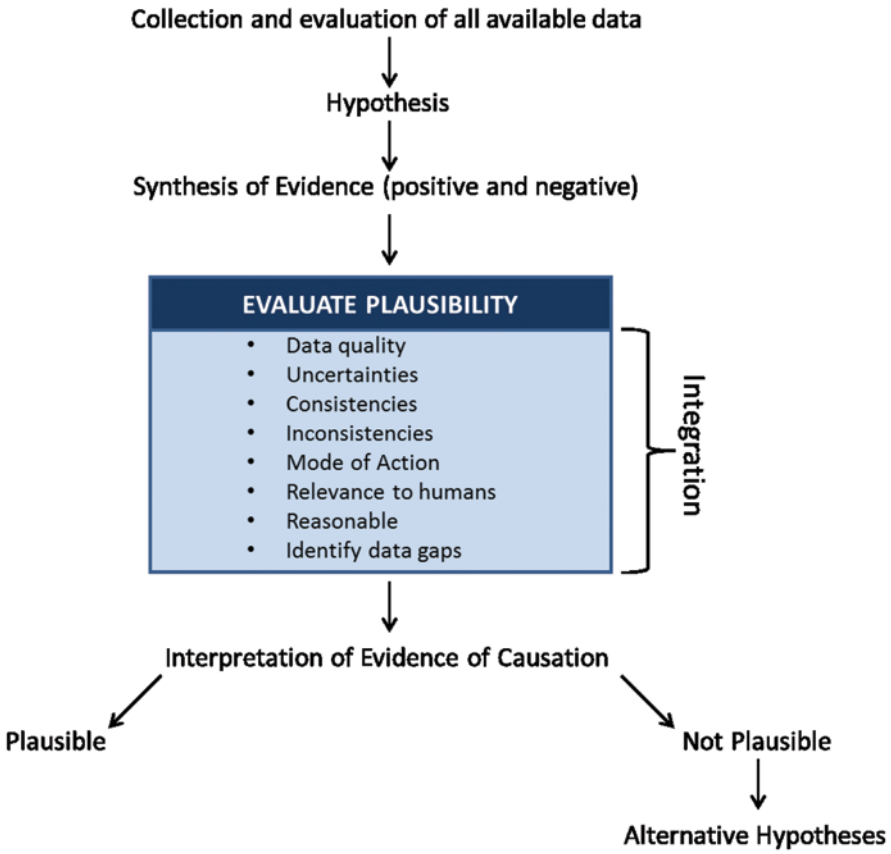

Fig. 2.3 Illustrates the process of assessing and integrating evidence during hazard identification. The integration of various lines of evidence is sometimes called a weight of evidence analysis wherein available data is evaluated to determine if exposure to a chemical of concern causes the observed adverse effect(s)

managers, policy makers, and other stakeholders to determine how to respond to a risk such as a chemical of concern. The WOE approach is advantageous because it provides an objective means of dealing with a diverse body of scientific data, which is particularly important when stakeholders involved in the process lack unity regarding the interpretation and application of available data (ECHA 2010).

Scientific data regarding chemicals of concern may come from a diverse array of sources. As discussed above, epidemiological studies, laboratory studies in animals or tissue culture models may form the basis for a hazard identification of a data rich chemical. However, other sources, which may provide less detailed information, may be of use in cases where little is known about a chemical of concern. Below are additional sources of information beyond standard scientific literature (e.g., PubMed):

Handbooks:

- American Conference of Governmental Industrial Hygienists

Databases, Quantitative Structure Activity Relationship Tools, and Commercially Available Software:

- OECD Screening Information Data Set (SIDS)
- National Library of Medicine TOXNET
- National Library of Medicine PubChem Project
- USEPA Aggregated Computational Toxicology Online Resource (ACToR)
- USGS CERC Acute Toxicity Database
- USEPA Integrated Risk Information System (IRIS)
- ATSDR ToxProfiles
- Provisional Peer Reviewed Toxicity Values (PPRTVs)
- European Commission IHCP Danish QSAR Database
- International Programme on Chemical Safety (IPCS INCHEM)
- International Toxicity Estimates for Risk (ITER) Database
- National Industrial Chemicals Notification and Assessment Scheme (NICNAS) Chemical Assessment Reports
- National Toxicology Program (NTP) Study Reports
- Risk Information Exchange (RiskIE) Database
- The Registry of Toxic Effects of Chemical Substances (RTECS)
- Toxic Substance Control Act Test Submission (TSCATS) Database
- read-across studies
- OECD QSAR Toolbox

In addition to there being data that must be synthesized from diverse sources, a WOE approach must find a means of prioritizing or weighting data to select which data are to be given the greatest significance. The outcome is a more consistent, scientifically defensible, and transparent approach to data evaluation. When data from diverse sources support the same conclusion, this alone leads to greater confidence on behalf of the evaluator that the outcome of the evaluation itself is scientifically sound (Suter and Cormier 2011). This luxury is not always available for chemicals

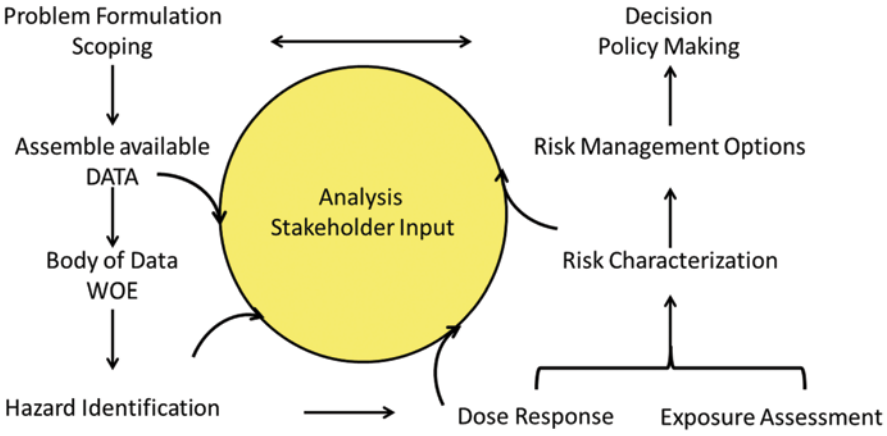

Fig. 2.4 Illustrates the process risk assessment and where hazard identification fits into that process. It is considered one of the earliest steps in risk assessment and includes the determination of whether a certain chemical poses a risk to human health. Decisions made during hazard identification are critical to determining whether a chemical-specific risk assessment will be conducted

with limited information or data. However, the goal of weighting data is to use the highest quality, most reliable data upon which to build a risk assessment that is fit for the purpose identified in the problem formulation and scoping phases (Fig. 2.4).

Product of the Hazard Identification Process

As above discussed, the overarching goal of the hazard identification (HI) step in risk assessment is to collect all available data, to evaluate that data, and to use some permutation of a WOE approach to formulate a conclusion about whether or not a chemical of concern causes an adverse health effect. Adverse health effects caused by a chemical of concern can be acute or chronic based on what exposure durations are investigated in the available body of data and what the intended human use for the chemical will be. Acute effects are those that are caused by a short duration of exposure (e.g., less than 24 h). Generally speaking, these effects are induced by higher concentrations of exposure and could include health endpoints such as respiratory and ocular irritation, odors, nausea, gastrointestinal effects, dermal irritation, hepatitis, et cetera. Often, acute effects are observed as the result of occupational or accidental exposure. Chronic effects are induced by long-term exposure, which is greater than 10% of the exposed organism's lifespan. In reality, chronic exposure in humans may last years to decades (e.g., epidemiological studies). For more information regarding basic definitions describing exposure, toxicity factor derivation, and risk assessment, the authors direct the readers to the USEPA IRIS Glossary (www.epa.gov/iris/help_gloss.htm) and the Texas Commission on Environmental

Quality's Guidelines to Develop Toxicity Factors (http://www.tceq.texas.gov/toxicology/esl/guidelines/about.html).

If a chemical receives HI classification as either an acute or chronic toxicant to one or more organ systems, this classifications has broad implications. For example, the citizens of California passed "The Safe Drinking Water and Toxic Enforcement Act of 1986", colloquially called "Prop 65". This law is designed to protect drinking water from toxic substances, particularly carcinogens and teratogens, by limiting businesses from releasing such chemicals into areas where they could contaminate drinking water. In addition, Prop 65 aims to reduce or eliminate public exposure via requiring businesses to add consumer warnings on product labels. This law is administered by the California Office of Environmental Health and Hazard Assessment (OEHHA). OEHHA conducts HIs to decide which chemicals are regulated under the authority of Prop 65. The HI assessment documents are publically available at the following link: http://www.oehha.org/prop65/hazard_ident/hazard_id.html. However, the liability of informing consumers falls to companies producing consumer products. Rather than labelling consumer products as containing toxic chemicals, most companies opt to reformulate their consumer products rather than risk civil litigations. The economic impact of this law on companies is considerable with a high potential for abuse and as a result this law remains controversial.

While the HI process itself appears simple on the surface, this critical part of the risk assessment process is highly complex. HIs outcomes can vary greatly among scientists. However, due to the regulatory and economic impact of HI, this step is sometimes controversial and influenced by policy and public opinion as can be seen in the following section discussing application of HI data.

Application of Hazard Identification Information

Although hazard identification information is used primarily to perform a risk assessment, the information also serves in describing the hazard potential (i.e., intrinsic toxic properties) that can be subsequently communicated to those in occupational settings or handling consumer products. This type of information becomes very useful in mitigating an unwarranted chemical exposure by encouraging appropriate handling and use of Personal Protective Equipment (PPE) in an occupational setting when needed or design of environmental controls aimed at mitigating exposure to a chemical agent. In the United States, the Occupational Safety and Health Administration (OSHA) require manufactures' of hazardous chemicals to inform users of any intrinsic properties by supplying this information on a Safety Data Sheet, formally known as the Material Safety Data Sheet. The new revised Hazard Communication Standard has adopted the Global Harmonization System (GHS) developed by the United Nations (Federal Register 2012). The intent is for the intrinsic hazards associated with a chemical agent to be uniformly communicated and applied based on a set criteria to be globally harmonized. Some countries, such as the European Union, have already adopted GHS by incorporating it into their regulation.

Chemical Management Programs sponsored by the Environmental Protection Agency and OECD have facilitated the gathering of various endpoints: physical chemical, environmental fate, environmental and toxicological data to assess the hazard potential of chemicals. In Europe, the REACH regulation requires manufactures' (registrants) to submit information in the form of data, such as toxicological studies, to describe the potential intrinsic hazards associated with the chemical agents. The data requirements vary based on the annual tonnage level or band (i.e., 10, 100, and 1000 t/year), but more importantly registrants are required to conduct a risk assessment and assign the appropriate hazard classification based on the data obtained (http://echa.europa.eu/regulations). These types of efforts have improved the communication of the intrinsic hazards of chemicals in commerce, and more importantly the information obtained has helped prioritize what chemicals need to be assessed and also those that should be considered for replacement. For example, the state of California has promulgated the California Safer Consumer Products (SCP) Regulation (DTSC 2013). SCP was created to identify and prioritize chemical agents of concern present in consumer products that may pose a substantial risk to humans. California Department of Toxic Substances and Control (DTSC) is the main driving force of the SCP. DTSC has defined a chemical of concern as exhibiting a hazardous trait and/or environmental or toxicological endpoint, and listed on one of 13 individual candidate-chemical watch lists. According to Cowan et al. (2014), the SCP regulation will have significant impacts on global consumer product manufactures and other responsible entities given the size of the economy in the state of California. These types of activities will only continue to gain momentum given the growing concerns with chemical agents posing a potential human health risk.

Evolving Trends in Hazard Identification

Toxicological data developed using standardized test protocols are best described as measurements of apical endpoints such as cancer, reproductive, developmental and neurotoxicity. These type of studies are costly and require an extensive period of time to gather this type of information on a chemical agent. More importantly, this approach does not provide sufficient detail to support a mechanism of action (MOA) and as a result is not typically incorporated into today's chemical risk assessments (Carmichael et al. 2007). Given the large amount of chemical agents today that lack sufficient data to determine whether they pose little or no concern, there is a growing awareness for the applicability of newer techniques such as High Throughput Screening (HTS) approaches to identify how chemical agents are interacting with biomolecular targets that may result in perturbations characterized as potentially deleterious (Dix 2007). Currently, programs such as ToxCast led by the U.S. Environmental Protection Agency are focused on utilizing *in vitro* assays that consistently identify alterations of biological processes of relevance to *in vivo* toxicity (Judson et al. 2010). This type of effort ties in well with the National Academy of Science (NRC) vision.

The intent with programs such as Tox21, ToxCast, and AXLR8 is to clearly modernize the current paradigm of toxicology testing by utilizing *in vitro* models to detect significant perturbations in cellular pathways (Stephens et al. 2012). Perturbations associated with a chemical agent at the gene or protein level should serve as a discriminatory tool to understand the mechanistic basis for what constitutes an adverse effect (Boekelheide and Campoin 2010). Tice et al. (2013) have provided an update regarding the progress made with Tox21 and noted the utilization of quantitative high-throughput screening approaches aimed at identifying and mapping biological perturbations associated with chemical agents has shown significant promise. However there are still matters that need to be sorted out such as incorporating metabolism using in vitro assay systems, assessing the effects of chronic exposure, and determining if a perturbation in a gene or pathway is associated with an adverse effect that is likely to be observed in animals or humans. More importantly as described by Thomas et al. (2012), the use of high throughput *in vitro* toxicity screening assays needs to provide significant predictive performance for both specificity and sensitivity for various *in vivo* endpoints of interest. The basic idea is use HTS to survey potential molecular initiating events (MIE) when looking to identify a toxic response from chemical agents. Clearly an understanding of the (MIE) and toxicology pathways associated with chemical agents at the gene or protein level will greatly improve our understanding of what constitutes an adverse versus adaptive response and be incorporated successfully into a risk assessment.

Building on the idea of (MIE) and Adverse Outcome Pathway (AOP) by utilizing these advanced technologies such as systems biology and high throughout assays is expected to improve our understanding of the toxicological pathways associated with various chemical agents (Slikker 2007). One example of the usefulness with these approaches is illustrated by Keller et al. (2012) with dimethylarsinic acid (DMA^V). Toxicology data developed on (DMA^V) using traditional toxicology tests showed transitional cell tumors of the urinary bladder in rats following chronic exposure in drinking water or diet. Using transcriptional profiling, Keller et al. (2012) described how Sen et al. (2005) demonstrated molecular changes in the transcriptome at doses below those previously used which showed transitional cell death in target epithelium evaluated among other apical endpoints. They also showed subcytotoxic doses resulted in a gradual progression with altered changes in gene expression occurring at the lowest level and irreversible changes in tissue responses with extended treatment in animals. The utilization of this type of information will be a significant improvement in terms of characterizing an adverse effect and the relevance to humans for risk assessments moving forward.

In Europe, the EU Framework Program 6 has utilized “omics” technologies in combination with traditional animal testing to investigate phenotypic changes at the gene, protein, or metabolite level (Suter and Cormier 2011; Suter et al. 2011). This approach has been useful in identifying putative biomarkers and development of mechanistic hypothesis that can be further investigated. Boiter et al. (2011) as part of this framework investigated the mechanistic basis associated with gene expres-

sion levels and liver hypertrophy. They demonstrated how transcriptomics could be used to differentiate responses characterized as adaptive by measuring increases in liver size that is necessary to accommodate a functional load attributed to activation of nuclear receptors that induce xenobiotic metabolizing enzymes or peroxisomal fatty acid β-oxidation necessary to maintain functionality of the liver. Although the examples described here show great promise for the use of these technologies, there are some factors that need to be taken into consideration on how this information will be utilized for the purpose of risk assessment and hazard identification. For example, the volume of data generated with these technologies and lack of a systematic review process for evaluating and interpreting the data needs to be standardized before the information can be used for conducting risk assessments (Pettit et al. 2010; Goetz et al. 2011). However, overall these new approaches hold great promise in characterizing what constitutes an adverse effect and more importantly will aid our efforts in establishing the relevance to humans.

The growing need to minimize the use of animals for testing and the REACH regulation in Europe has facilitated the utilization of read-across or category approach to characterize potential hazards associated with a substance or group of substances deemed to have similar toxicological and physical chemical properties (ECETOC 2012). The concept behind read-across is to utilize existing information available for similar chemistries to draw conclusions about the potential toxicity with a similar chemical. The similarity between chemistries can be described by some of the following: having common functional groups (aldehyde, epoxide, or specific metal ion), being part of the same chemical class or similar carbon number ranges, and similar metabolism or by-products. There are a number of references developed by the OECD, European Chemicals Agency (ECHA), and ECETOC that the reader is encouraged to follow-up with to learn more about the utilization of read-across (OECD 2007; ECHA 2010; ECETOC 2012).

A workshop including representatives from industry and regulatory agencies was recently held to share on past experiences using read-across and identify areas where read-across approaches could be improved (Patlewics et al. 2013). Although the use of this approach is promising, there still remains matters related to determining how best to build on scientifically valid and robust justifications to gain acceptance for this approach, and that the characterization reflects the hazard potential in a conservative manner without being unrealistically conservative.

An example on how read-across can been successfully applied comes from Yamada et al. (2013). They investigated approaches to categorize various allyl esters with the potential to cause repeated dose hepatotoxicity. The culprit associated with the liver toxicity is attributed to the metabolism of the allyl alcohol to acrolein that has been shown to be cytotoxic to the liver. The authors were able to develop a data matrix to build a category in order to predict whether other allyl esters types based on their metabolic profile were likely to cause hepatotoxicity. The framework described by Wu et al. (2010), have also demonstrated the use of chemical and biochemical principles with an emphasis on metabolism (bioactivation) to identify and evaluate the suitability of various analogues for the purpose of read-across. As

noted by Patlewics et al. (2012), toxicokinetic information is useful in drawing conclusions about the potential for certain chemicals being read-across or in a category to describe the manner by which they may elicit a toxic response.

The successful utilization of read-across also relates to the level of understanding regarding the MOA. For example, much effort has been placed understanding the molecular initiating event (MIE) associated with skin sensitization (OECD 2012). Substances that can cross the epidermis have the ability to bind to proteins that are then internalized by Langerhan cells and transported to the draining lymph node. The covalent binding of a substance, whether metabolically activated or not, has been characterized as the key initiating event and has been further characterized as an adverse outcome pathway (AOP) for skin sensitization. The AOP is a conceptual framework allowing one to draw a linkage between a MIE and adverse outcome. The key to an AOP is that the MIE has to be anchored to endpoints that are of specific concern related to risk. By understanding these events, scientists are convinced the AOP of a particular endpoint can be used to address issues related to interspecies extrapolation in terms of better understanding points of convergence and divergence within pathways and the extent these pathways are conserved across taxa of interest (Ankley et al. 2010).

Summary

Hazard identification continues to be an important step in the risk assessment process, and the learning objectives outlined at the beginning of this chapter were intended on orienting the reader to those items needing consideration. Although this chapter was never intended on being overly comprehensive, the information described should provide explanations on how Hazard Identification is utilized in the context of a risk assessment. In addition, sufficient detail and types of resources have been provided to assist the novice risk assessor in identifying the information available and criteria to use when evaluating the database to establish a potential adverse effect associated with a chemical agent. This chapter also introduces the importance for understanding mode-of-action (MOA) associated with a chemical agent to understand its relevance to humans and also provide a description associated with an observed adverse effect. Further characterization of MOA will only continue to improve with the utilization of high throughput screening assays and "omic" applications aimed at identifying toxicological pathways associated with perturbations in normal cellular function. This type of mechanistic insight will facilitate our understanding of the nature of the toxicity associated with chemical agents and should also improve the risk assessment process. Granted these efforts are a shift from the current paradigm with traditional toxicology testing, but once refined should provide the necessary information to aid in prioritizing chemical agents with the greatest risk of exposure and impact on human health.

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Chapter 3
Dose-Response Assessment

Thomas A. Lewandowski and John Norman

Abstract The goal of dose-response assessment is to quantitatively describe the relationship between the extent of exposure (the dose) and the likelihood of adverse health effects (responses). The outcome of dose-response assessment is a value which, when combined with the exposure estimate, will allow an estimation of health risk. Key tasks in dose-response assessment are the compilation of dose-response data from animal and human toxicology studies, selection of the most sensitive and convincing health effects or endpoints, identification of a suitable no or minimal effect dose associated with those effects, and selection of uncertainty factors and dose-response models that can be used to derive a safe level for human exposure.

Keywords NOAEL · BMD · Slope factor · Dose-response curve · Threshold · RfD · RfC

Student Learning Objectives

After studying this unit, you should be able to:

- describe what is a dose
- describe how dosage data should be graphically represented
- define and describe LD50, NOAEL, LOAEL, BMD
- identify LD50, NOAEL, LOAEL, BMD on a graph
- describe a threshold and a non-threshold model and when each is appropriate to use

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Purpose of a Dose-Response Assessment

The fundamental principles of toxicology rely on the understanding of the causal relationships between exposure and effect. The father of toxicology, Paracelsus (1493–1541) recognized the importance of this relationship and famously stated, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy." In risk assessment the dose–response assessment phase characterizes the relationships between varying doses and the degree of effect in a population (humans, experimental animals) exposed to the substance(s) in question. Exposure to a low dose of a chemical may produce no effect (no risk) or may be beneficial in the case of essential nutrients. As the dose increases, detrimental effects may start to be observed and at high enough doses, death may occur. It is therefore important to understand this relationship in order to estimate potential health risks.

Dose-response assessments are critical to toxicologists, regulatory agencies, occupational health professionals, and health safety managers. In order to limit the risk to the population, exposure to an agent should be limited to a "safe dose" or the dose at which there is minimal risk. In public health, the dose-response relationship is used to drive regulatory limits for a wide range of concerns including: air, water and soil concentrations; food additives and contaminants; hazardous waste site clean ups, consumer product safety, and warning labels and restrictions. The **ultimate goal** of dose-response assessment is to explain the relationship between exposure to an agent and adverse effects and to reasonably protect the public from that effect. Whereas hazard identification (Chap. 2) aims to determine whether an agent can cause adverse effects (a qualitative, yes/no type of question), dose response assessment tries to understand this relationship quantitatively; how the relationship between exposure and effect changes with the magnitude and duration of exposure as well as other factors affecting susceptibility (*e.g.*, age). In practice, these two stages of risk assessment represent a continuous evaluation process and studies and assessments designed for hazard identification typically also attempt to provide quantitative information about dose-response relationships.

Introduction to Dose Response

Introduction to Dose

The term dose can be misleading. The average person is most familiar with the term dose when taking medication, i.e. one adult tablet of aspirin is 325 mg. In this sense, the dose is the amount of agent coming into contact with an organism or a part of an organism. However, there are important considerations associated with "dose" that should be accounted for in order to completely characterize the potential effect of an agent. The *administered dose* is the amount of an agent introduced

to an organism (*e.g.*, eaten in food, inhaled in air, *etc.*). The *absorbed dose* is the actual amount of an agent coming into contact with the body's internal tissues and may only be a fraction of the administered dose (*e.g.*, a portion of the ingested dose may pass unabsorbed through the GI tract). Total or cumulative dose is the sum of all individual doses administered to an organism over a given time. In many experiments, administered dose serves as the default metric of exposure because of a lack of information about the absorbed dose. In such a case, a default assumption is made that the percent of the dose absorbed (*i.e.*, the bioavailability) is 100%. In other cases, the extent of absorption can be determined (usually through a separate bioavailability study) and the administered dose can be converted into an absorbed dose. This latter approach may be undertaken when there is reason to believe that the extent of absorption may be well below 100% (*e.g.*, for some metal compounds with low solubility) and/or when small changes in risk estimates (*e.g.*, due to differences between administered *versus* absorbed doses) may have significant impacts on environmental cleanup decisions.

A condition providing an opportunity for an external environmental agent to enter the body is an exposure. Exposure to an agent can occur through food or water intake (oral route), application of an agent to the skin (dermal route), through the air (inhalation), or through medical intervention (parenteral route). The exposure route is a critical consideration when evaluating the dose of an agent and the effect it may have on an organism.

Dose in toxicology is typically measured in the same way as in medicine and in pharmacology. The units commonly used are the gram (g), milligram (mg), or part per million (ppm). The amount of an agent must be related to the organism receiving the dose. A typical method of standardizing and comparing doses is to relate the amount of an agent to the organism's body weight in kilograms (kg or kg bw). One of the most frequently used dose measurements for oral or dermal administration is mg/kg, or milligrams per kilogram of body weight. The amount of time over which a dose is administered is another critical criteria often represented in the dose unit. Most doses are standardized to a day (d), but hours (h) and weeks (w) can also be used. The final dose unit may therefore be expressed as mg/kg-day or mg/kg bw-day. For exposure to an agent through the inhalation route, it is typical to see dose represented as mg per volume of air expressed as cubic meters (m^3), liters (L), or parts per million (ppm) or parts per billion (ppb).

Introduction to Response

From a practical standpoint, the body's response to a dose of an agent is the question toxicologists are trying to answer. A small amount of an agent may be required and even beneficial to an organism where higher doses can cause adverse effects. Iron is one such example. A deficiency leads to anemia in adults causing fatigue and impairing the ability for adults to do physical work. It is important humans consume enough iron to avoid this deficiency. The daily Tolerable Upper Intake Level of iron for adults is 45 mg/day and is the maximum dose an adult should take per day

(Institute of Medicine 2010). [Note this dose has not been adjusted for body weight; the average adult human is typically considered to weigh 70 kg and the body weight adjusted dose is 0.64 mg/kg-day]. At higher doses, iron is corrosive to the gastrointestinal tract and toxicity for adults begins at doses above 20 mg/kg-day. As the dose increases, the severity of the response increases as well and 60 mg/kg-day is considered an acutely lethal dose (Spanierman 2013). In this example, the magnitude and type of response is strongly correlated to dose.

Toxicological responses are physiological effects due to the administration of an agent, as illustrated above. Responses occurring at the area of the body where the agent was applied or administered are called local effects. Dermal injuries following the application of acid or lung tissue damage due to the inhalation of a reactive gas are examples of local toxicity effects. After an agent has been absorbed and distributed throughout the body from the entry point, systemic effects may be observed. Systemic effects occur at locations other than at the entry point. Systemic effects will strongly depend on the absorption, distribution, metabolism and elimination (ADME) of the agent in the body. For example, some agents will be preferentially distributed and stored in certain tissues where they may exert a toxic effect (*e.g.*, lead and the central nervous system, benzene and bone marrow). Metabolism is also critical, because some agents are only toxic once metabolized to reactive products (*e.g.*, some insecticides, benzo(a)pyrene) whereas for other agents, metabolism is a means of reducing toxicity (*e.g.*, other insecticides, arsenic). Because metabolism varies across both individuals and species, understanding how metabolism affects toxicity is an important part of dose-response assessment.

It is not uncommon for an agent to adversely affect one organ more than another once an agent is absorbed into the body (*e.g.*, the liver is often in this category due to its predominant role in toxicant metabolism). Target organ is the term used to describe the tissues that exhibit the major toxic effect in the body. The central and peripheral nervous system, liver, kidney, lungs, the hematopoietic system, skin, and the reproductive system are common target organs.

Timing of Responses to Toxic Agents

Toxicologists and risk assessors are also concerned about the time involved between exposures and the appearance of adverse effects and consider both acute and chronic toxicity. Acute toxicity occurs immediately or nearly immediately following exposure. Acute exposures are typically a single dose or several doses occurring within a day. Animal experiments with acute exposure are primarily concerned with lethality. Experiments with oral and dermal exposures use the lethal dose: 50% (LD_{50}) or the dose causing death in 50% of the animals, as the primary measurement; inhalation exposures use the lethal concentration causing 50% mortality or LC_{50}. The subscript value represents the percent of the population exhibiting the response (LD_{10}, LD_{50}, LD_{75}, etc). In an occupational setting, narcosis, disorientation, and death are often of greatest concern. Other commonly used measurements for

acute exposures include: the effective dose or concentration (ED_{50} or EC_{50}) eliciting a response in 50% of the individuals and the TD_{Lo} or $TC_{Lo,}$ the lowest published toxic dose or concentration. Tests for determining acute lethality have been standardized (*e.g.*, OECD 425 and 403) and traditionally have been the most widely available information for individual chemicals. However, because lethality is too drastic an endpoint for evaluating human safety, and due to animal welfare concerns, tests focused on acute lethality are becoming less common and are being replaced with acute toxicity tests focused on more subtle endpoints.

Authoritative bodies have set limits for acute exposures. For example, for the workplace the American Conference of Governmental Industrial Hygienists (ACGIH) and the American Industrial Hygiene Association (AIHA) have developed STELs (Short-Term Exposure Limits), concentrations no person should be exposed to for more than 15 min during an 8-hour workday, and ceiling values, concentrations no person should ever be exposed to during the work period (ACGIH 2013; AIHA 2013). For the general public, other acute exposure values such as the Emergency Response Planning Guidelines (ERPGs), Acute Exposure Guideline Levels (AEGLs) and Temporary Emergency Exposure Limits (TEELs) have been established by various groups. These values typically take a tiered approach, with the lowest tier values (*e.g.*, AEGL-1) designed to prevent any adverse effect (*e.g.*, irritation) whereas the highest tier (AEGL-3) are intended to protect against lethality or permanent injury.

Repeated exposure over the course of several weeks or months can lead to subchronic toxicity. Exposure to an agent continuously or repeatedly for a significant portion of the lifespan can lead to chronic toxicity. Both subchronic and chronic toxicity tests are primarily concerned with systemic toxicity of a particular organ(s). Animal experiments designed to examine toxicity from subchronic or chronic exposures are very detailed; most tissues in the animal are examined for macroscopic and histopathological leasions to look for toxicity. Subchronic tests often serve as screening tests and are typically conducted over 28-day and/or 90-day periods. A one to two year bioassay is considered to be a chronic toxicity test and is most often used where there is a concern an agent may be a carcinogen, often based on preliminary findings in the subchronic test.

Because toxicology studies may not always correspond to the exposure period of interest, risk assessors sometimes have to infer a relationship between dose and response over different periods of exposure. For example, an acute animal study may involve an exposure of only 4 h but a risk assessor may want to develop a safe exposure limit for people exposed for 24 h. Although extrapolating dose-response relationships across time frames involves some uncertainty, there are methods for doing so. For example, Haber's Rule postulates that dose and time of exposure can be mathematically related as follows:

$$Dose \times time = k$$

where k is constant that is specific to the chemical and health effect in question. Thus if a given level of effect (*e.g.*, 50% CNS depression) occurs at 50 mg/m^3 for

30 min and k = 1, then for 60 min a similar level of effect can be expected at a dose of 25 mg/m^3. Most toxicologists are cautious in applying Haber's Rule as it may not produce valid results if used to extrapolate far beyond the timeframe of the original data. Uses of Haber's Rule for extrapolations to very short or very long timeframes (less than 10 min or more than 48 h) have not been validated with test data.

Dose Response Curve

The dose-response relationship is the most fundamental, essential, and pervasive concept in toxicology (Klassen 2013). It relates exposure to an agent and the spectrum of effects caused by the agent. Information about the effects of an agent can be determined from human observational studies, animal studies, or studies conducted in cell culture (*in vitro*). Recent advances in toxicology and in computational modelling have enabled scientists to determine dose-response relationships purely *in silico*. The dose-response relationship can be represented graphically, also termed a dose-response curve.

The dose-response curve can represent the response of an individual or the response of different individuals in a population. A dose-response curve for an individual typically shows the gradation of response in an individual as they are exposed to increasing amounts of an agent. The effect of increasing doses of alcohol in an individual is one such example. A dose response in this case would document the response spectrum beginning with no effect and then moving through successive

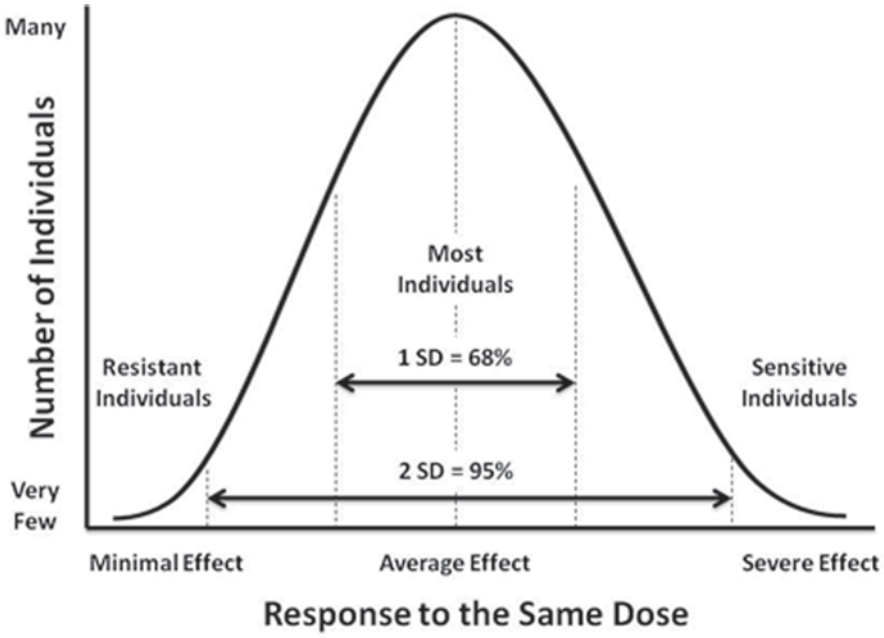

Fig. 3.1 Example of variability in response to a given dose within a population

degrees of inebriation towards death as the dose increases. While this type of dose-response is interesting, toxicologists are usually concerned with the distribution of responses in a population of individuals.

The dose response curve has its basis in population statistics and generally is expected to approximate a normal or Gaussian distribution (Fig. 3.1). The normal distribution curve represents the range of responses to the same dose in a population of individuals. The severity of effect to the same dose is plotted along the x-axis and the frequency of the effect in the population is plotted along the y-axis. Variability within the population will result in a range of response. Age, weight, gender, metabolic (gene) variations, and current health status are only some of the variables contributing to population variability. The mean response is the average effect exhibited by the majority of the individuals in the population. The standard deviation (S.D.) shows how much variation from the average exists. For a true normal distribution, one S.D. +/− mean covers 68.3 % of the population; two and three S.D. +/− the mean covers 95.5 and 99.7 % of the population, respectively. This type of dose response curve is especially useful in medicine when determining if there is a particularly sensitive subpopulation of individual to a drug. When the frequency of response is converted to cumulative response and the dose is varied, a sigmoidal (S-shaped) curve is observed.

Concepts of Dose-Response

The sigmoidal curve is the classic shape exhibited by most dose-response curves (Fig. 3.2). Experimental data are typically plotted with the dose or concentrations on the x-axis. The units for the dose or exposure depend on how the experiment was

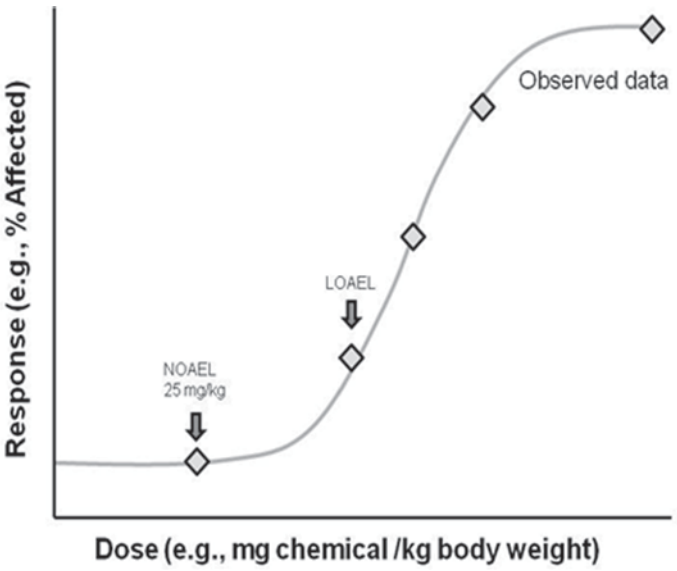

Fig. 3.2 Example of a classic sigmoidal shaped dose response curve

conducted as discussed above. The cumulative response is represented on the y-axis and is usually expressed as percent of the total population. As shown in Fig. 3.2, the dose response curve is drawn as close as possible to the individual data points. Note that a given agent will have many dose-response curves, one for each toxic effect of interest.

The level at which a toxic effect is first encountered is known as the threshold dose. Below the threshold dose there are no adverse effects from exposure to the chemical. This dose is also called the no observed adverse effect level or NOAEL. In Fig. 3.2, 25 mg/kg-bw is the threshold or NOAEL. The human body attempts to maintain a stable environment or homeostasis. When a toxicant is introduced into the body, the body attempts to detoxify and eliminate the agent in order to avoid damage, and failing that to repair any damage caused. The threshold dose represents the point beyond the body's ability to detoxify the agent and/or repair the damage. The lowest observed adverse effect, or LOAEL, is the first dose where an adverse effect is observed. As the dose increases, more of the population is affected and exhibits signs of toxicity until all, or mostly all of the individuals are affected.

The shape of the dose-response curve is important for determining the relative potency of an agent. The potency of an agent is a measure of how toxic the agent is compared with other chemicals. The greater the potency of the agent, the lower the amount required to cause a response. The slope, or the percent of population responding per unit change in dose is one way to determine the potency of an agent. Typically the slope is measured at the central portion on the dose-response curve. A steep curve beginning at a low dose indicates the agent is likely to have a high potency. For acute studies, the lower the LD50 an agent has, the higher the potency is. Figure 3.3 illustrates this principle. Even though Toxicant A has a higher threshold than Toxicant B in Fig. 3.3, Toxicant A has a lower LD50 and a steeper slope indicating Toxicant A is more potent on a population basis than Toxicant B. [Note

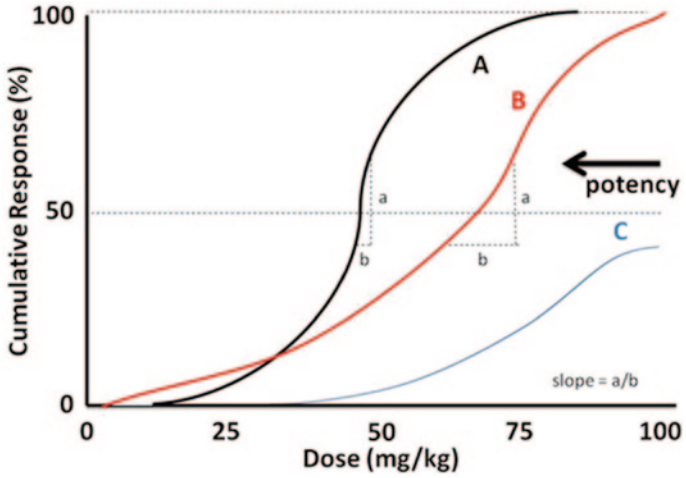

Fig. 3.3 Examples of dose response curves with differing slopes and potencies

Toxicant C has a high threshold and very shallow slope indicating it is the least potent of the three toxicants].

The LD_{50} is focused on the use of lethality as a measurement for toxicity however, toxicologists are concerned about other adverse effects to the population. Adverse effects resulting from toxicant exposure can physically present as nausea, rash, dizziness, headache, hair loss, loss of sensation in the arms and legs (peripheral nervous system), learning disabilities, bone density, etc. These effects may present rapidly or may develop over the course of a life-time, increasing the difficulty in demonstrating causation between an effect in humans and an agent. Regardless of the adverse effect being examined, the dose-response curve is a useful tool to describe and visualize the relationship between dose and response.

Dose Response Assessment for Noncancer End Points

A Brief History and the Use of Threshold Dose Curves in Regulation

The history of the dose-curve and its use to protect public health has its roots in the Federal Drug Administration (FDA). Prior to the passage of the 1906 Federal Food and Drugs Act, food and drug regulation was either non-existent or was poorly regulated by the states. With the passing of the 1906 act, the FDA required accurate labelling of ingredients used in food and prohibited the sale of any drug not conforming to the concentration and purity specifications in the US Pharmacopoeia and the National Formulary without proper disclosure. Despite the new authority and some initial successes, the FDA still lacked the regulatory and legal tools it needed to adequately protect the public. In 1938, the Food, Drug, and Cosmetic Act (FDCA) was passed in response to a medical disaster when the drug sulfanilamide dissolved in diethylene glycol, an industrial solvent, killed over 100 people. The FDCA required manufactures to demonstrate their drugs were safe to the FDA before the drug could be sold. The FDCA also tightened up food safety regulations and required color additives to be certified as harmless by the FDA for use in cosmetics.

After the passage of the FDCA, toxicologists searched for a method to limit exposure to pesticides, food and color additives commonly used in commerce to ensure the public's health. Scientists at FDA's Division of Pharmacology published a series of articles entitled "Procedures for the Appraisal of the Toxicity of Chemicals in Foods" that helped to standardize the tests and procedures required to demonstrate the safety of a chemical. These reports culminated in a publication by Arnold Lehman and O. Garth Fitzhugh that served as the foundation for the acceptable daily intake (ADI) (Lehman and Fitzhugh 1954).

An ADI is the maximum amount of a chemical considered safe to ingest each day for the duration of a lifetime. The ADI is based on a threshold concept; while all agents are toxic at high enough doses, there is a dose where no adverse effects

are observed in a large population. As long as the threshold is not exceeded, then the agent can be consumed safely and pose no significant risk. An ADI is developed by identifying the appropriate no effect level or NOAEL in animal toxicity studies, and applying safety factors to ensure public health is protected. Thus:

$$ADI = \frac{NOAEL}{Safety\ Factors}$$

The selection of safety factors will be discussed further in the following section. It is important to note the threshold model does not apply to some carcinogens (as discussed in Sect. 4).

Points of Departure

Toxicologists typically rely on data from animal studies to set values such as ADIs and RfDs. Because the number of animals used in such studies is limited, the doses used are typically much higher than normal human exposure levels to be sure of observing possible adverse effects (an issue of statistical power). The value obtained from the animal study which serves as the starting point for dose response assessment is the Point of Departure (POD), as described below.

No Observable Adverse Effect Level/Concentration (NOAEL or NOAEC) A NOAEL is defined by EPA as, "[a]n exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control. Some effects may be produced at this level, but they are not considered as adverse, nor precursors to adverse effects." If the lowest dose or concentration of an agent tested in an experiment still demonstrates adverse effects, the dose is the Lowest Observed Adverse Effect Level/Concentration (LOAEL or LOAEC). Where several NOAELs and LOAELs are reported for different endpoints, regulators focus on the highest NOAEL that is still below the dose where adverse effects were observed (LOAEL), leading to the common usage of the term NOAEL as the "highest exposure without adverse effect" (U.S. EPA 2001).

Establishing Toxicity Criteria Once N(L)OAELs and N(L)OAEC values have been identified in the available studies, they can then be used to establish chronic toxicity criteria. Common chronic criteria include the reference dose (RfD) and reference concentration (RfC) established by US EPA, the acceptable daily intake (ADI) mentioned previously (typically used for chemicals in the diet), and the tolerable daily intake (an analogous term more commonly used outside the US). Each of these values is derived in a similar fashion. The RfD and RfC differ primarily by the concentration units they are expressed in with the RfD being expressed in mg/kg-day and the RfC being expressed in mg/m^3 air. These values are established by considering data from toxicology studies (typically animal studies) and applying appropriate uncertainty factors (also called safety or assessment factors). The RfD

and RfC are intended to be estimates "(with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime." (US EPA 1999).[1]

Traditionally, these values were obtained by reviewing all of the available toxicity data and selecting the NOAEL/LOAEL for the most sensitive effect (*i.e.*, the one occurring at the lowest dose). This value then becomes the POD. Uncertainty factors are then applied to account for variability within the human population (interindividual or intraspecies variability) and variability between humans and the test species (inter species variability) if applicable. Additional factors might be applied as appropriate to adjust for use of data from a shorter term study (*i.e.*, subchronic to chronic exposure), use of a LOAEL rather than a NOAEL (if one has not been determined), and possibly due to limitations in the database (*e.g.*, a lack of data on carcinogenicity or reproductive and developmental effects). Thus:

$$RfD\,or\,RfC\,or\,ADI\,or\,TDI = \frac{NOAEL\ (or\ LOAEL)}{UF1 \times UF2 \times UF3,\,etc.}$$

More recently, it has become increasingly common to replace the NOAEL/LOAEL as the point of departure with the use of a benchmark dose or benchmark concentration approach (BMD or BMC). In a seminal review by Kimmel and Gaylor (1988), the limitations of using the N(L)OAEL in risk assessment were identified. The identification of a N(L)OAEL is limited to the specific doses tested and the slope of the response has little to no role in determining the N(L)OAEL. Insufficient animal sample size in experiments can result in larger than expected N(L)OAELs. Finally, the determination of a N(L)OAEL requires scientific judgment and is often the source of controversy. In contrast, the BMD approach incorporates all the dose-response data within a study not just the lowest dose. As a result, some view the BMD as more accurate than the use of a N(L)OAEL. The US EPA now advocates for the use of the BMD method in instances where environmental agents may cause health effects in exposed populations (US EPA 2014a).

The BMD or BMC approach involves mathematically fitting a dose-response curve to all the study data and then uses that curve to identify an estimate of the dose corresponding to a predetermined adverse effect (the benchmark response or BMR). Typically, dose-response curve fitting programs are used with the selected curve (*i.e.*, mathematical function) being the one that yields the best statistical fit to the data. The adverse effect level of response is typically between 1 to 10%, depending on the power of the study. The Benchmark Dose Lower-confidence Limit (BMDL or if a concentration BMCL) is the statistical lower confidence limit of the dose at the BMD or BMC. The BMD or BMDL is then combined with the appropriate set of uncertainty factors as described above to arrive at the toxicity criteria. For example:

$$RfD = \frac{BMDL_{05}}{UF1 \times UF2 \times UF3,\,etc.}$$

[1] US EPA, 1999. Reference Dose (RfD): Description and Use in Health Risk Assessments Background Document 1A March 15, 1993. http://www.epa.gov/iris/rfd.htm.

The US EPA developed the Benchmark Dose Software program (BMDS) to apply BMD methods to hazardous pollutant risk assessments (US EPA 2014b). The software includes a detailed tutorial and is a good resource for becoming familiar with the use of BMD methods, see Chap. 11 on resources for more information.

Concerning the uncertainty factors applied to the POD, these are traditionally applied for a series of different concerns or areas of uncertainty in translating the POD data into a safe exposure level for a human population. These concerns are:

1. Extrapolation between results of animal studies and the human population which is to be protected. A default assumption in risk assessment is that humans are more sensitive than the animal test species. A value of 10 is usually applied as a default to characterize this difference in sensitivity. However, if studies or models suggest greater or lesser sensitivity between humans and the test species this uncertainty factor can be made more case specific. For example, mathematical models of metabolism (physiologically-based toxicokinetic models, PBTK) have been used in some instances to quantify human-rodent differences in the metabolic production of reactive metabolites (Clewell et al. 2005). Models of the rodent and human airways have also been used to develop specific dosimetric extrapolation values for certain reactive gases (Jarabek 1995). This particular uncertainty factor has been thought of as equally representing toxicokinetic differences (*i.e.*, ADME) and toxicodynamic differences (cellular and molecular mechanisms) across species with a subfactor of 3 assigned to each.
2. Extrapolation for intra-individual differences in susceptibility. This factor is meant to address differences such as age, gender, health status and genetic makeup which may render individuals more or less sensitive to a toxic insult. Because animal studies are generally conducted on in-bred strains of adult animals with very little genetic variability, the default value of 10 is rarely modified. Exceptions would be cases where the population of interest is composed only of healthy adults or where the POD is obtained from a study focusing on a sensitive group (*e.g.*, epidemiology studies of methylmercury's neurodevelopment effects measured in children).
3. Extrapolation between study duration and human exposure period. The third uncertainty factor relates to the duration of the study which generates the POD. Most frequently, risk assessors are interested in protecting people from chronic, lifetime exposures so data from a chronic toxicity study are most appropriate. Lacking this, data from a subchronic (*e.g.*, 90-day) study may be used. In this instance, however, an uncertainty factor of 1 to 10 may be used to "adjust" for the difference in exposure times. Note that this factor is only used when chronic study data are not available.
4. Extrapolation between a NOAEL and LOAEL. In cases where a NOAEL value cannot be identified and a LOAEL must be used instead a factor of 1 to 10 is often used to ensure the final value results in no adverse effect.
5. An uncertainty factor may also be applied when data are lacking about a particular endpoint of interest (*e.g.*, developmental toxicity, neurological toxicity) that may be of concern for the agent in question. This was formerly described as a

"modifying factor" or "database uncertainty factor." Although a maximum value of 10 may be used, a value of 3 is also commonly seen.

Combined, these 4 uncertainty factors have a maximum value of 10,000. Such an extreme case is rarely found however, because in such a situation quantitative dose-response assessment is probably not advisable. It is more commonly the case that a combined UF of between 100 and 1000 is used. These still represent a very great decrease in the dose obtained from the actual toxicity study.

Cancer

What is Cancer and How Does it Occur?

Cancer is a disease that arises from the loss of control over normal cell replication. Unlike the case with the health effects described above, in cancer tissues are not damaged *per se* but rather abandon their normal functions and focus exclusively on replicating. Because cancer cells do not carry out their normal activities, tissues lose their functional capacity. The expansion of non-functional cancer tissue may also adversely affect what functional tissue remains.

Cancer results from damage to the cell's genetic material (DNA) and most importantly, the particular genetic material controlling cellular replication. Specific cellular genes (*e.g.*, p53, VHL, APC, FasR) normally prevent the cell from undergoing runaway replication. Damage to these genes, or interference with their products, can result in cancer. This alteration can occur in two ways. First, the cell's DNA can be directly damaged by interaction with chemicals (*e.g.*, those with highly reactive functional groups) or energy (*e.g.*, ultraviolet light, X-rays). This damage, if unrepaired, can lead to the loss of control over cellular replication and, eventually, cancer. Alternatively, repeated tissue damage or some other trigger (*e.g.*, an implanted insoluble body, perturbation of a hormone signal or interference with gene transcription) can cause sustained cellular proliferation as a response. This situation increases the likelihood that critical genes will be incorrectly copied and lead to a loss of cellular control over replication.

These two pathways to cancer are reflected in two different risk assessment models of the carcinogenic process. The first, the genotoxic model, is probabilistic in nature. This means that any DNA damage event has the potential to lead to cancer, assuming (1) the damage was in a critical gene, (2) the damage is not repaired before the normal function of the gene is needed, and (3) that the damaged cell survives the body's surveillance system for detecting and eliminating damaged cells. Given these caveats, any DNA damage event can produce cancer, but the probability per single event is extremely small. This is demonstrated by the fact that while a typical mammalian cell sustains approximately 10,000 "DNA hits" each day (largely due reactive chemicals created during normal metabolism), most cells do not progress

to cancer (Ames et al. 1993). Nonetheless, each DNA damage event is theoretically capable of producing cancer and there is no threshold. From a risk assessment standpoint, there is no safe dose but only a dose which can produce a negligible increase in the probability of developing cancer (*e.g.*, 1 in a million increased risk).

In contrast, the second carcinogenic model involves a period of prolonged proliferation due to some perturbation or damage. However, the body posses an inherent ability to resist such effects *e.g.*, the ability to detoxify exogenous agents at low levels of exposure or to repair low levels of insult without producing a sustained proliferative response. Thus, in this risk assessment model there is a threshold below which the risk of cancer is zero. This is analogous to the conceptual process for non-carcinogenic health effects discussed in Sect. 3. These two different views of cancer influence the way dose response assessment is carried out in risk assessment.

Animal Studies to Assess Carcinogenic Potency

While initial studies of potential carcinogenicity can be traced to the early 1900s in studies of coal tars and coal tar products such as benzene (Yamagiwa and Ichikawa 1915; Selling 1916) it was not until the 1940s that a test protocol to detect potential carcinogenicity was generally discussed. Scientists at the US Food and Drug Administration (FDA) published "Procedures for the Appraisal of the Toxicity of Chemicals in Food" (FDA 2005). In FDA's protocol, two species (typically rats and dogs) were tested for 2 years in 4 different dose groups: control, probable no effect level, mid-dose group and high-dose group. After 2 years of chronic exposure, all tissues were saved, preserved and evaluated for tumor incidence. Similar methods were published in 1958 by the World Health Organization (JECFA 1958). The FDA protocol was refined in the late 1960s by scientists at the US National Cancer Institute (NCI) led by John and Elisabeth Weisburger. The NCI published detailed recommendations for a standard cancer detection protocol with studies conducted in both rats and mice, use of oral gavage dosing, and carrying out the experiment to 92 weeks of age in mice and 104 weeks of age in rats (Weisburger and Weisburger 1967; Weisburger 1999). At least 3 dose groups were used to better characterize the potency and dose-response pattern of the carcinogen. The top dose, the maximum tolerated dose (MTD) was one that fell just short of causing apparent toxicity during the dosing period (*e.g.*, a greater than 10% reduction in body weight). The idea was to ensure the greatest likelihood of observing a carcinogenic response in a limited number of animals. Use of the MTD has been criticized for decades as being unrealistic and perhaps not indicative of carcinogenic potential at much lower levels of exposure. The NCI also identified specific strains of animals for testing (F344 rats and B6C3F1 mice) focusing on those with a low background rate of tumor incidence. This protocol, dating from the late 1960s remains the gold standard today, and has been given an OECD test designation of 451. The 2 year bioassay is typically supplemented with a host of supporting studies that help to identify the proposed dose levels (initial acute and subchronic studies), and the possible mode(s) of action for carcinogenesis (*e.g.*, metabolism studies, genotoxicity assays, shorter than lifetime studies that look for signs of precursor events).

Dose-Response Assessment for Carcinogens

In the US and some other countries, genotoxic and non-genotoxic carcinogens are evaluated differently in terms of the dose-response extrapolation. The approach for genotoxicants is to take data from an animal bioassay (or rarely, an exposed human population) and use modeling to establish a dose-response curve. There are multiple dose-response models that have been used to perform dose response analysis for cancer. Some (*e.g.*, the probit, logistic and Weibul models) are purely statistical whereas others (*e.g.*, the one hit model, the multistage model) have a basis in concepts about how the carcinogenic process occurs. For example, the multistage model contains mathematical terms that are intended to correspond the discrete events in the carcinogenic process (*e.g.*, the initial DNA damage, failure of repair, proliferation of the mutant cell type, *etc.*). The specific model chosen is typically the one that best fits the observed study data (determined *via* statistical measures such as the Maximum Likelihood Estimate).

The most commonly used approach, the linearized multistage model, involves drawing a straight line downward from the upper confidence limit at the lowest datapoint (or a modeled incidence rate such as 5%) to the origin. Thus the model assumes that zero cancer risk only occurs in the complete absence of exposure. The 95% lower confidence limit on the slope of the dose-response model in the low dose range (*i.e.*, in the range of possible environmental exposures) is used as the cancer potency factor (CPF, formerly called q1*). This is equivalent to the increased cancer risk per mg/kg-day of exposure. [Note that unlike the non-cancer approach described in Sect. 3, this approach does not apply specific factors to account for interspecies and intraspecies variability; the inherent assumption is that the low dose extrapolation to risk per mg/kg-day of exposure is sufficiently conservative to account for such factors]. The CPF can then be multiplied by an exposure estimate to arrive at a cancer risk.

It is not always certain that the linear approach best describes the data as other extrapolations (sublinear, supralinear) may also be possible and may be consistent with different theories of the disease process (Fig. 3.4). Because high dose range data provide little indication of the best form of extrapolation in the low dose region, there is often disagreement among experts about how extrapolation should be conducted. Within the low dose region, the risks predicted by these models can vary over several orders of magnitude (Krewski et al. 1990). Even so, the linearized multistage model has been adopted by US regulatory agencies as well as some groups outside the US. For example, the World Health Organization (WHO) uses the linearized multistage model and a risk target of 10^{-5} in order to set guidelines for carcinogenic chemicals in drinking water (WHO 1998).

The approach for *non-genotoxic* carcinogens is similar to that for non-carcinogens discussed in Sect. 3. The dose-response data from an animal or human study are used to define a threshold below which no increased incidence of cancer is anticipated. Either a NOAEL or BMDL value may be used. More practically, the assessment may focus on a precursor element of the mechanistic chain of events producing the cancer (*e.g.*, hormone perturbation, cell hyperplasia), which can be more readily observed. This threshold, when combined with appropriate uncertainty factors, then becomes the toxicity criterion.

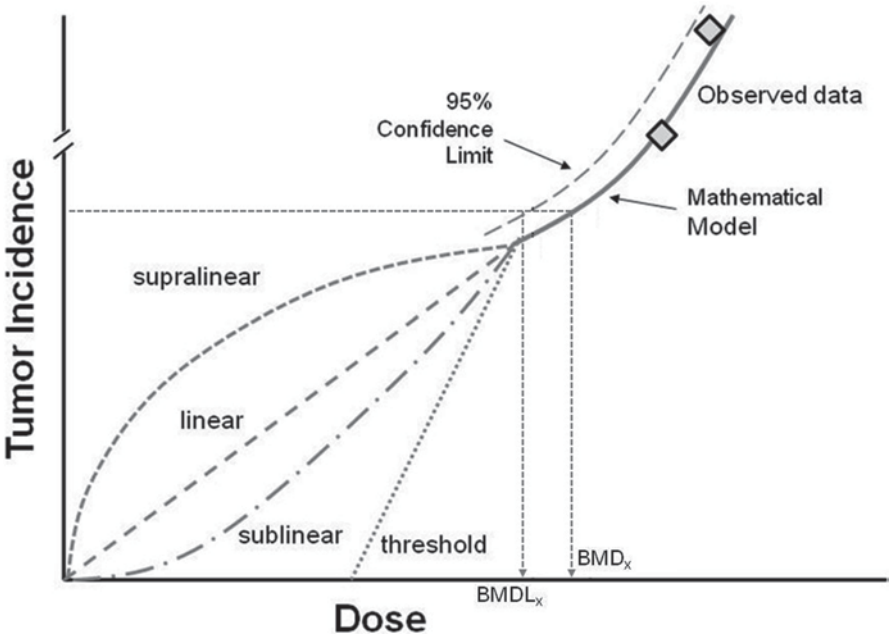

Fig. 3.4 Different models for conducting low dose extrapolation

This dichotomy in approach has attracted considerable criticism. The idea that any dose is associated with a definite if low level of cancer risk is inconsistent with the body's known ability to protect against and repair DNA damage (recall the 10,000 hits per cell per day mentioned earlier). There is therefore probably a practical threshold for all carcinogens but identifying that threshold with certainty is difficult. Most carcinogens act by multiple modes of action, some genotoxic and others non-genotoxic. For example, trichloroethylene and tetrachloroethene can both cause direct DNA damage (by formation of several possible reactive intermediates, *e.g.*, divinyl cysteines) but also *via* non-genotoxic proliferation (*e.g.*, *via* the peroxisomal proliferation receptor PPAR) (US EPA 2011, 2012). The relative importance of each pathway appears to be both species and tissue dependent. Thus, the difficulty becomes determining which mode of action is predominant. Because of limitations in animal studies, it is rarely possible to conclusively demonstrate that one mode of action is exclusively the important one and as a result, regulators typically default to the genotoxic mechanism which yields the most conservative risk estimate. Recent reviews of the risk assessment process by the US National Research Council have identified development of a unified framework for assessing all types of chemical effects as a priority for improving the process of risk assessment (NRC 2009).

In Europe, some regulatory agencies have not adopted dose-response modeling as a regulatory approach. These regulators view the tumorigenic dose (TD) as a useful way of ranking or comparing the carcinogenic potencies of different

chemicals. The TDx is used to describe the doses that produce an x% incidence of tumors in an animal study. For example, the European Chemicals Agency (ECHA) uses the TD25 with adjustments for spontaneous background tumors and survivability (fewer animals at higher doses may develop tumors but that is because they may have died). The TD25 is converted to a human-specific dose factor (HT25) by dividing by appropriate allometric scaling factors (default: [animal weight/human weight]$^{0.25}$) to account for species differences in metabolism. An estimated human exposure is then divided by HT25/0.25 (0.25 being a standard coefficient to relate the 25% incidence level to a NOAEL value) to give an estimate of the possible human cancer incidence.

An alternative approach is taken by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA). This approach uses a Margin of Exposure (MOE) methodology to avoid the uncertainties associated with quantitative dose-response extrapolation to exposure levels well below the range of animal dosing. The MOE is a ratio of the no observed effect level to the level of anticipated exposure. Thus,

$$MOE = \frac{NOAEL \ or \ BMDL}{Expected \ Exposure}$$

The approach yields an aggregate margin of exposure or safety; *i.e.*, the width of separation between a threshold dose for health effects and the daily dose expected in the population of concern. The MOE is a simpler estimate of risk that can be more easily explained to the public. The question then becomes how large the MOE must be to be considered acceptable (a policy rather than a science decision). In general, the EFSA considers that an MOE of 10,000 or larger indicates that chemical exposures are of low health concern (EFSA 2004). This value of 10,000 is based on the assumption that the two 10-fold factors for inter- and intra-species differences used for non-carcinogens are also relevant here but that an additional factor of 100 is required for carcinogenic endpoints. This latter factor takes into account that the BMD estimate is not equivalent to a NOAEL, additional uncertainties are involved related to intra-human variability in control of cell division, DNA repair, and the shape of the dose–response curve below the BMD (EU 2008).

Under the European REACH regulations, chemical manufacturers and importers are required to calculate Derived Minimal Effect Levels (DMELs) for non-threshold carcinogens. The European Chemicals Agency (ECHA) has published guidance indicating that either a linear extrapolation or margin of exposure (here called a "large assessment factor") approach may be used and leaves the choice up to the individual assessor (ECHA 2010). Thus the approach for estimating cancer potency is determined by the specific regulatory program involved. ECHA DMELs are treated more comprehensively in Chap. 9: Risk Assessment in the European Union.

Dose-Response Assessment as a Component of a Risk Assessment In integrating dose-response information into a risk assessment, it is important that the relevant data be described in a clear and coherent manner. While many toxicologists

engage in risk assessment, not all risk assessors are toxicologists and the details of dose-response assessment may not be inherently obvious. In order to increase transparency and confidence in the dose-response portion of the risk assessment, it is recommended that information be organized in the following steps:

Step 1. The practitioner should carefully review and summarize all of the relevant toxicological data for each chemical of concern. For animal studies, the includes the study hypothesis (the question being addressed), the species and strain of animals used, dosing protocol, observational methods (*e.g.*, tissues analyzed), results (including statistical analyses) and any issues or limitations noted by the study authors. For human studies, the review should describe the study hypothesis, the population(s) being studied, the magnitude and duration of exposure (including how the exposure was determined), the methods of analysis, the study conclusions and any limitations or problems (*e.g.*, confounding variables) described by the authors. Supporting information (*e.g.*, *in vitro* studies, physiological models, mechanistic studies) should also be reviewed and subjected to critical evaluation in terms of how well they support the results of the animal and human data.

Step 2. Based on the thorough review, the risk assessor should determine the weight of evidence (WOE) for each adverse effect of concern. Similar to a WOE analysis for hazard identification, this will involve assessing study quality, representativeness and validity (Lewandowski and Rhomberg 2005).

Step 3. Based on the WOE evaluation, the risk assessor should then select the endpoints where data are clearest and where sufficient data exist for dose response assessment. For carcinogens this means the specific type of tumor with the best support in terms of dose response information and consistency across different studies. For non-cancer endpoints, this means looking at different organ effect data and selecting those that are most sensitive as well as those which have sufficient data for dose-response modeling. Several options should be selected if BMD analysis is intended to allow the assessor to choose the endpoint with the best overall model fit. Note that the requirement for good and clear dose-response data may produce a different result than the WOE analysis conducted for hazard identification.

Step 4. Discuss the POD approach to be used (NOAEL or BMD) and justify the choice in a transparent manner. It may be that data are lacking to carry out a BMD analysis whereas the traditional NOAEL approach requires only a single NOAEL or LOAEL value.

Step 5. If dose-response modeling is used, the modeling process should be described in a transparent and easy to replicate manner. This includes providing the datasets used as input to the modeling, the modeling program used and the criteria used to select a particular dose-response function. If more than one function seems plausible, the different options and the reason for choosing one over another should be discussed.

Step 6. The specific uncertainty factors applied to the POD to derive the final value should be discussed. The risk assessor should explain why each factor was or

was not included and justify the particular value assigned for each factor. This might require a particularly detailed discussion for the interspecies factor if modeling or other studies are being used to describe species differences in kinetics or dynamics. Consider using appendices to provide supporting data and preventing the reader from getting lost in the details.

Step 7. Finally, the risk assessor should present the final toxicity criterion (RfD, RfC, ADI, TDI, CSF or TDx) and discuss any uncertainties that exist and how they might affect this value. For example if certain health endpoints have not been studied but some relevant information is available for similar compounds this could be discussed as part of a "what if" scenario. The impact of decisions made during the dose response assessment in terms of modeling choices or uncertainty factor values should be discussed where relevant.

Emerging Issues

As with other parts of risk assessment, the field of dose-response assessment is developing in light of new technologies and new regulatory pressures. As noted above, current tests to identify the potential chronic toxicity of chemicals are time consuming and very expensive. As a result, only a small fraction of chemicals currently in use have been fully tested. In addition, new chemicals are continuously being developed. The REACH regulations in Europe (and similar laws adopted or being considered in other countries) require that complete information be developed for all chemicals in commerce. It is recognized that a full *in vivo* set of study data for each unstudied chemical would be extremely expensive, time consuming and would completely exceed existing testing capacity. Society is also becoming less open to the idea of animal testing and one additional element of REACH is that it requires that testing in animals be minimized wherever possible (EU 2003). As a result, there has been renewed interest in developing *in vitro* and *in silico* methodologies that can be used to greatly reduce, if not replace, whole animal studies. The first task is to validate such tests against existing whole animal data to demonstrate their predictive ability. It is likely that a whole host of assays will be required to carry out this function.

In 2007, the U.S. National Academy of Sciences published the document "Toxicity Testing in the 21st Century (TT21C): A Vision and a Strategy" (NRC 2007). The document lays out a pathway towards taking advantage of available advances in molecular technology and computational systems biology to streamline and accelerate the process of toxicity testing and reduce the number of animals used. A major focus is on using omics technology to develop a better understanding of the disease process. The goal is to use these technologies to elucidate toxicity pathways that chart relationships between the various molecular and cellular events contributing to the disease outcome. This goes beyond the more general concept of mode of action in that it examines the relationship between a large number of genes and their products and the interaction stemming from their up and down-regulation. Once the

toxicity pathway is understood, batteries of assays that evaluate individual pathway components can be designed and implemented to predict whether a given chemical can cause perturbation of normal function. This has potential to be a revolutionary shift in thinking—from simplistic tracking of specific adverse outcomes (tumors, reduced organ function, changes in cell population) to actually understanding how disease occurs. The Tox21 report calls for work on a set of example toxicity pathways (*e.g.*, those related to perturbation of estrogen signaling) to be conducted to build public confidence in the process. This will involve adoption of high throughput assays using human derived cells to investigate specific pathway elements. Bioinformatics tools will need to be developed (or borrowed from other fields, *e.g.*, pharmaceuticals) to allow the large amount of data generated to be integrated and evaluated. The NRC report acknowledges that it may not be possible to use *in vitro* and *in silico* systems to completely replace the need for *in vivo* testing (at least in the short term). The hope however is that these new techniques may be used to prioritize chemicals for testing and to more carefully allocate available whole animal testing resources.

Another emerging concern relates to the toxicology of mixtures. Although humans are exposed to broad mixtures of chemicals in combination rather than individual chemicals in isolation, the field of toxicology has focused on studying the health effects on chemicals in isolation for over 60 years. Although changes to this approach have been proposed since the late 1980s (Yang et al. 1989; Lewtas 1989), there has been an unwillingness to conduct toxicity studies of chemicals in combination. This is because studies of chemicals in combination would be of necessity far larger in scope (requiring far more dose groups to distinguish joint effects from individual effects) and require complex interpretation. Nonetheless, increasing pressure on regulatory agencies to address the issue of multiple chemical exposures has lead to increasing calls to study such concerns. In addition to chemical exposures, it is also being recognized that other factors (*e.g.*, stress) can exacerbate the effects of chemical exposures (Cory-Schlecta 2005; Cory-schlecta et al. 2008). Establishing protocols to incorporate these non-chemical factors into chemical testing will constitute a challenge in the coming decades. It is also important to note that the potential for chemical interaction (antagonism, synergism, potentiation) is likely to vary along the dose response curve. It is highly likely that interactive effects may occur at high doses (where, for example, natural detoxification or damage repair mechanisms are already overwhelmed) but may not occur at lower doses (where these mechanisms may be sufficient to negate the effects of multiple chemicals) (Lewandowski 2011). Again, this calls into question the usefulness of the high dose bioassay and argues in favor of toxicity pathway based approaches which may be more capable of detecting precursor effects at low doses and, due to their lower cost and higher throughput, be able to incorporate a larger number of dose combinations. The mixture chapter covers toxicology of mixtures in more detail.

Another approach for dealing with the large data acquisition requirements imposed by regulations like REACH are the related concepts of the Threshold of Toxicological Concern (TTC) and the so-called “read across methodology”. While not new, the TTC (Munro et al. 2008) has seen increasing use in both the US and Europe

as a way to speedily, if conservatively, assess health risks of chemicals in foods or cosmetics prior to the development of a full toxicological dataset. Under the TTC, chemicals are placed into groups based on structure activity relationships. Generic dose-response criteria are established for each group based on conservative assumptions, such as taking the 95th percentile lowest NOAEL of all chemicals in the group and dividing by a factor of 100. These criteria can then be applied to chemicals where data are lacking. For example, for genotoxic compounds (*i.e.*, those expected to be genotoxicants based on the presence of specific structural groups present in demonstrated genotoxicants) the TTC is 0.15 μg/person/day. For non-genotoxic compounds, the TTC varies by chemical class. The TTC approach specifically excludes certain compounds (metals, metal-containing compounds, polyhalogenated dibenzodioxins, dibenzofurans or biphenyls and proteins) because these are recognized to be either highly variable and requiring chemical specific assessment (*e.g.*, metals) or already have established dose-response methods (*e.g.*, dioxins and the toxic equivalency factors). The Read Across approach is similarly based on quantitative structure activity analysis (QSAR) and aims to interpolate dose-response data for chemicals with well established datasets to make predictions for chemicals where data are lacking. This again represents a practical effort to develop data to answer the requirements of regulations like REACH despite the limitations of existing testing capacity. The Read Across concept is covered in greater detail in the hazard identification chapter.

One concept that is not new but remains a subject of continued discussion is the idea of hormesis. The hormesis theory assumes that small doses of chemicals may actually have a beneficial effect in the body by stimulating metabolism or other detoxification mechanisms (Calabrese and Baldwin 1998). It is only when dose levels exceed the dose promoting stimulation that adverse effects can occur. The analogy is often made to vitamins and other essential nutrients that exhibit a “U shaped” dose response curve; *i.e.*, positive effects a low doses, neutral effects at somewhat higher doses (*i.e.*, not more positive but also not negative) and then negative effects as the dose increases beyond a certain level. Acceptance of hormetic activity would have only a limited effect on dose-response assessments for non-carcinogens, which are based on an observed threshold for adverse effects, but would profoundly affect the linear extrapolation conducted for carcinogens (Fig. 3.5). To date, support for the hormesis theory has been limited because detecting clear hormetic effects in whole animal studies has been difficult. As newer information is gained about cancer development pathways, it may be easier to document hormetic effects (*e.g.*, at the level of gene regulation).

The burgeoning field of nanomaterials represents another area where dose-response assessment will have to accommodate new technologies. Nanomaterials have distinct chemical and toxicological properties from their macroscale counterparts such that established information about the latter cannot reliably be used to make inference about the former. Thus whole new suites of testing will be required which will need to address novel questions. It is clear that nanoparticles' special properties stem from their small size and large surface to volume ratio. What then is the most relevant dose parameter: mass (as with bulk materials), particle size,

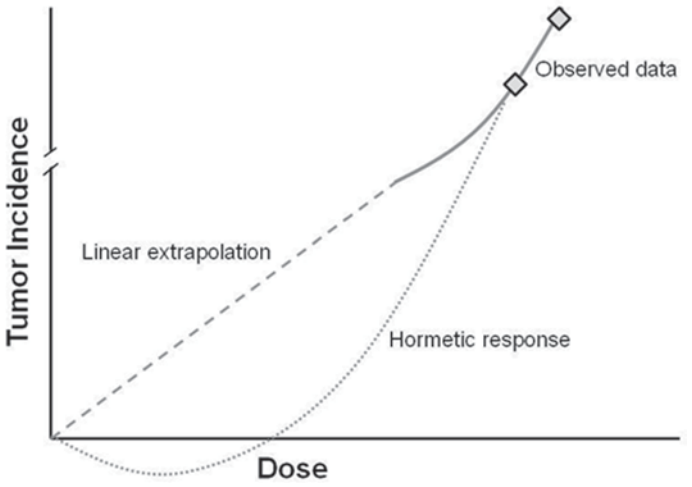

Fig. 3.5 Conceptual dose response curve involving hormesis

surface area, charge *etc.*? Clearly conducting studies so as to evaluate each property would be laborious and expensive. In addition, because nanomaterials are capable of penetrating tissues to a far greater degree than bulk materials, what different toxicological endpoints should be evaluated? It is also likely that nanomaterials differ substantially from one another not only on their component material (*e.g.*, carbon, titanium dioxide) but also their shape and size. All of these questions are currently being given careful consideration.

Chapter Summary

The dose-response curve is an important tool for understanding the relationship between exposure to an agent and its effect. Experimental results can yield critical information on the potency and the type of effect (acute/chronic, local/systemic, and non-genotoxic/genotoxic carcinogen) an agent may have on a population. The route and duration of exposure along with the absorption, distribution, metabolism and elimination (ADME) of the agent helps to inform the shape of the dose-response curve.

Ultimately, toxicologists and public health professionals will incorporate the dose-response curve into a risk assessment to establish regulatory limits to protect the public. In addition to the dose-response relationship, the potential for exposure and the natural variation in the population should be carefully considered and the appropriate safety factors applied to the point of departure. The emergence of new technologies and materials presents a challenge to classic risk assessment techniques, but the dose-response relationship will still serve as a key consideration in protecting the public's health.

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Chapter 4
Exposure Assessment

Thomas Grumbles

Abstract The risk that an employee faces on the job is a function of the hazards present and the actual exposure level to those hazards. This chapter covers how exposure evaluation is accomplished and how exposure evaluation has changed dramatically over the last 40 years. Sampling technology, exposure assessment strategies and limitations of historical exposure data use in risk assessment activities are reviewed.

Keywords Exposure assessment · Personnel monitoring · Air sampling

Student Learning Objectives

After studying this unit you should be able to:

- Understand the principles of occupational exposure monitoring methods
- Understand occupational exposure monitoring strategies
- Understand the uses and limitations of occupational exposure sampling results
- Be able to critically evaluate monitoring results and studies for strengths and limitations for use in risk assessment

Introduction

The risk that an employee faces on the job is a function of the hazards present and the actual exposure level to those hazards. How the hazards are determined and how the exposure evaluation is accomplished has changed dramatically over the last 50 years.

Risk assessors by necessity will generally need to rely on historical exposure data to provide the exposure element to any risk assessment activity. Therefore it is

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important to have a working knowledge of how exposure assessment data has been and is now generated. Furthermore, understanding the rationale used for determining assessment strategy and practices, the technologies and its limitations used to gather data and information, and strengths and weaknesses of the data will aid the risk assessor in completing their work.

The use of a systematic method to characterize workplace exposures to chemical, physical and biological agents is a fundamental concept driving occupational exposure assessment today but comprehensive exposure assessment methodology has not been the most common method of exposure assessment found in historical records or reports. In general exposure assessment and monitoring strategies in the past were not developed with future use in risk assessments in mind.

Occupational exposure assessments are often performed by individual's trained in Industrial Hygiene. It should be noted that the term Occupational Hygiene is also commonly used around the world and in this chapter these terms are used interchangeably. Industrial Hygiene is commonly defined as (DiNardi 1998):

> ..the art and science dedicated to the anticipation, recognition, evaluation, communication and control of environmental stressors in, or arising from, the workplace that may result in injury, illness, impairment, or affect the well being of workers and members of the community

One should not underestimate the "art" term in this traditional definition. Many early hygienists were working in a time when the sophisticated sampling instrumentation and methods available today did not exist. Early efforts in defining the occupational environment grew from traditional public health and air pollution methodologies.

The factors driving occupational exposure assessment in the last 50 years are multiple and varied. Understanding the nature of exposure monitoring in the past, the variables impacting data quality and subsequent utility of the data is an important element in developing risk assessments. Risk assessments are clearly impacted by the quality and accuracy of the exposure data used.

In general it is not commonplace to find historical occupational exposure data sets based on a statistical approach to providing representative information for an individual employee, group or facility, and it is even less likely to find datasets or studies that represent a particular industry subsector or group of facilities. For example, while for risk assessment exposure data for a group of workers that was generated in a statistically random fashion is preferred, "worst-case sampling" or monitoring the workers with the highest potential exposure during a short sampling campaign or period of days has been a more common industrial hygiene practice. Understanding these variables and how they impact the use of available exposure data is a key concept for risk assessors (OPPT, USEPA 1994).

Information in this chapter will provide an overview of occupational exposure assessment, exposure monitoring strategy, general sampling approaches and some detailed information on the technical aspects of monitoring methods. Taken together this is meant to educate the reader in the use of exposure data in the conduct of risk assessments.

Exposure Assessment and Monitoring Defined (Plog 1996; DiNardi 1998)

Employee Exposure is a potential exposure to chemical, physical or biological agents that occurs in the workplace regardless of the use of personal protective equipment.

Exposure Assessment is the qualitative or quantitative determination made by an industrial hygienist or other appropriately trained individual of an employee's exposure to a chemical, biological or physical agent.

Quantitative Exposure Monitoring is the direct measurement of employee exposure using direct reading instrumentation or sample collection for analysis. Monitoring, or sampling, is usually done by drawing a measured volume of air through a filter, sorbent filled tube, impingement device with collection media or other instrument to collect the airborne contaminate of concern.

Qualitative Exposure Assessment is an assessment and possibly ranking of workplace hazards based on a collection of information and observations including frequency, magnitude, and variability of exposure and tasks. There are currently multiple defined systems and approaches to accomplish documented qualitative assessments.

Sample in the context of exposure monitoring is typically meant to reference an airborne contaminate collected by sampling methodology described above (Quantitative Exposure Monitoring). This term can create confusion as the word "sample" can be construed to describe a collection of several items with common characteristics that are evaluated.

Exposure assessment in the workplace has been practiced for many years. However the advent of quantitative exposure monitoring practices and workplace exposure data development were to a great extent driven by the establishment of the Occupational Safety and Health Agency in the early 70's and the regulations regarding workplace exposure that followed. In the last 50 years the "art and science" of exposure monitoring have seen significant advancements thus improving the ability of Industrial Hygienists and others to more accurately and in many cases cost effectively, measure workplace exposures.

In general exposure assessments will include documented qualitative assessments, modeling techniques, and quantitative measurements.

Qualitative Exposure Assessment

Qualitative exposure assessment may be used initially to estimate or determine potential personnel exposures at or above levels of concern. This assessment can define quantitative assessment needs or determine that no further action is needed. This determination can be made by an occupational hygienist or other trained persons that are familiar with the operation or process being evaluated. Initial evaluations could lead to the decision that there is insufficient information available for

an immediate determination of exposure potential. Further information gathering or quantitative sampling may be required to assess exposures.

Occupational exposure depends on the characteristics of the substance of concern, products processes, tasks/work activities, and exposure control or risk management measures in place. Qualitative assessments often include gathering information on the following elements:

- A description of operations, tasks, or processes, including work practices and procedures, frequency and duration of operation and may include a diagram of the work area.
- Developing a list of all potentially hazardous materials used, stored, handled, or produced.
- Developing a description of how the chemicals are used, amounts in inventory, and estimated consumption rates.
- Developing a list of potential physical hazards, such as noise, heat, ionizing and non-ionizing radiation.
- Developing a list of potential biological or infectious agents.
- Direct reading screening measurements for each work area where applicable.
- Description and efficiency of existing controls. This includes the type of personal protective equipment (PPE), administrative controls, and engineering controls in use and evaluations of their effectiveness.
- The number of personnel assigned to each work operation/process (total, male and female).
- Frequency and duration of specific tasks that are associated with exposure potential.

It is possible to create a semi-quantitative ranking of exposure scenarios by assigning a number or other scaling indication to key parameters such as hazard/toxicity, frequency of tasks, length of exposures, etc. This can provide a documented relative measure of exposure potential across job categories or other groupings of workers.

For the purposes of risk assessment a documented qualitative exposure assessment can identify potential exposures for the population of concern, identify frequency or intensity of exposure potential and possibly rank overall exposure potential. In general these assessments will not provide a quantitative exposure level.

Exposure Modeling Techniques

In recent times the need for exposure assessments in the workplace has grown rapidly. This has been driven by multiple regulatory needs and the advent of a more comprehensive exposure assessment strategy approach by many in Industry. This need has resulted in the refinement of existing and development of multiple new exposure modeling techniques. These models are currently in use by multiple government entities and industrial personnel and have developed over time with increasing capability to provide quantitative estimates of exposure. For instance, early models provided screening and exposure range levels while more recent models provide a

relatively finite exposure level calculation for use. Input parameter flexibility, standard assumptions for release conditions and factors for exposure control measures in place have been added over time. Much of the modeling capability has been focused on inhalation exposures but the dermal exposure modeling functionalities have been improving steadily. These tools have also become more user friendly and readily available for use from Internet resources.

In general the available models are by design conservative in the outcomes and many feel they tend to overestimate exposures. Exposure models can be categorized into various tiers. In general models with a higher tier are more complex and realistic models. The literature will often refer to First Tier and Second Tier models. Some work has been done over time to correlate models results versus exposure measurements of the same operations. Although promising, more work is necessary. It is difficult to make a general conclusion on the accuracy and precision of modeling results compared to what measured exposures may indicate for any given worker or activity. Knowledge of the specific models used to report exposure results is needed to determine the overall utility of the information.

Selected Models Descriptions

EASE

The Estimation and Assessment of Substance Exposure (EASE) model has been under development and in use since the early 1990s. The UK Health & Safety Executive (HSE) developed the EASE model in collaboration with the UK Health & Safety Laboratory (HSL) as a general model to assist in exposure assessment for both new and existing substances and to be applicable to a wide range of substances and circumstances of use.

EASE can be used to predict inhalation and dermal exposure using task and situation specific information about the substances and methods of control. For inhalation exposures, the model predicts a range of expected exposures for the given set of circumstances. The dermal exposure model predicts the potential exposure of the hands and forearms expressed as a mass per unit area of exposed skin per day.

European Center for Ecotoxicological and Toxicology Of Chemicals (ECETOC) Targeted Risk Assessment (TRA)

The TRA tool contains methodologies developed to estimate inhalation and dermal worker exposures. The tool is also provided in an integrated version, which allows the user to perform worker, consumer or environmental assessment via one interface.

For occupational exposure the ECETOC approach uses established exposure prediction models (EASE with documented modifications by industry experts) but introduces a more precise, structured and simplified approach in order to make

it amenable to a more rapid assessment and to a wider user community. The approach also uses the common practice in the workplace that, by using a suitably conservative exposure prediction model which leads to a demonstration of low risk for a specific scenario of use, the subsequent need to collect and use measured exposure data for another assessment of the same scenario is minimized.

The concept for the worker exposure function was to provide the user with the risk assessment methodology that selects the Process Categories (PROCs) for the broad sector of use (either industrial or professional) of a substance, and then enables further modifications by selecting exposure control (Risk Management) measures that will impact the exposure levels. The ECETOC TRA for workers is considered a First Tier tool. It is therefore intentionally limited in scope and detail.

The input parameters for ECETOC TRA worker exposure calculations are:

- Molecular weight (needed for recalculation from ppm to mg/kg bw/day and for the recalculation to mg/m^3)
- Physical state of the substance (solid or not)
- Vapour pressure (liquids/gases) or dustiness (solids) category
- Process Category (PROC) as defined in the program
- Whether the activity is industrial or professional
- Whether the activity takes place indoors or outdoors
- Presence of Local Exhaust Ventilation (LEV; only for indoor activities)
- Duration of the activity (in ranges)
- Type of respiratory protection used
- Whether the substance is used in a mixture
- Concentration range of the substance in the mixture (in ranges)

EMKG-Expo

The exposure prediction model of the German EMKG-Expo-Tool9 (“Easy-to-use workplace control scheme for hazardous substances”) is a generic tool that can be used to derive a Tier 1 inhalation exposure value for the workplace (EMKG, BAuA 2008). The tool was developed to help small and medium sized companies to comply with the Chemical Agents Directive. The EMKG-Expo-Tool is based on the banding approach of the Control of Substances Hazardous to Health (COSHH) Essentials originally developed by the UK Health and Safety Executive (HSE). While the COSHH Essentials tool is seen as a qualitative approach to guide the assessment and management of workplace risks, the EMKG-Expo-Tool can also be used as a generic tool for assessing and comparing the level of exposure with limit values (OEL, DNEL). Hence the EMKG-Expo-Tool should be seen as an approach for filtering the non-risky workplace situations from those requiring detailed attention.

The EMKG-Expo-Tool uses the following input parameters:

- Volatility or dustiness,
- Amount of substance used
- Control strategy

- Exposure controls in place
- Exposure period (< 15 min or > 15 min)

The tool predicts a lower and an upper value for the exposure range (in mg/m^3 for solids and ppm for vapours).

United States Environmental Protection Agency (USEPA) ChemSTEER

The USEPA has developed the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER) to evaluate occupational and environmental exposures. ChemSTEER estimates occupational inhalation and dermal exposure to a chemical during industrial and commercial manufacturing, processing, and use operations involving the chemical. The tool estimates releases of a chemical to air, water, and land that are associated with industrial and commercial manufacturing, processing, and use of the chemical.

The tool allows users to select predefined industry-specific or chemical functional use-specific profiles or user-defined manufacturing, processing and use operations. Using these operations and several chemical-specific and case-specific parameters and general models, the ChemSTEER computer program estimates releases and occupational exposures. The methods in ChemSTEER were developed by the EPA Office of Pollution Prevention and Toxics (OPPT); Economics, Exposure, and Technology Division; Chemical Engineering Branch.

Input parameters for the tool include:

- Understanding of processes (for operations without industry-specific data) and of ChemSTEER methods (in HELP screens).
- Data and information on a chemical's:
  - Physical-chemical properties, including molecular weight, vapor pressure, and density.
  - Production or use volume, and if applicable, fractions devoted to multiple uses.
  - Weight fractions and physical states.
- Case-specific parameters, when available:
  - Numbers of sites, operating days, and workers; batch amounts and durations.
  - Release sources and worker activities.
  - Workplace concentrations and release amounts and media.
  - Types and sizes of containers used to transport the chemical or mixture.

While measured exposure data is preferable in the conduct of risk assessments the advent of exposure assessment models has presented a new tool for all concerned when working to develop the exposure portion of any risk assessment. These models have a varying degree of precision or accuracy. They can be easily obtained and run with most personal computers. There is still additional validation work to be done for some models but the results are generally accepted and can be reliably utilized in the right context and understanding of their limitations.

Quantitative Monitoring Strategies (Conrad and Soule 1998; Gross and Morse 1996)

There are multiple reasons for doing occupational exposure monitoring. The purpose driving the exposure monitoring activity will impact the depth and scope of the assessments. Regulations in many countries now mandate some periodic review of exposures throughout an organization. While these regulations are highly variable in scope, detail and enforcement, the trend is clear; more regulation has driven more monitoring. The activity may be part of a comprehensive exposure assessment strategy or a random activity driven by annual schedules or response to workplace events. It is not the norm to find studies of occupational exposure based on a statistical approach to providing representative information for an individual facility, and it is even less common to find exposure data sets that represent an entire category or classification of workers. Some general approaches to monitoring are described below.

Compliance Monitoring

A significant portion of the occupational exposure data in the United States has been generated to satisfy legal obligations of various types. The most common evaluations are conducted to compare measured exposure levels to established Permissible Exposure Limits (PELs) or meet the requirements of substance specific Occupational Safety and Health Administration (OSHA) regulations. Regulatory PELs are established by federal or local, such as state, agencies. Other established exposure limits can be determined by non regulatory bodies such the American Conference of Governmental Industrial Hygienists (TLV®s) or the American Industrial Hygiene Association (WEEL®s).

OSHA substance specific standards specify monitoring strategy and frequency but are only established for a small number of chemicals. These include lead, asbestos, benzene, inorganic arsenic, ethylene oxide and other chemicals regulated as carcinogens by OSHA. Although these OSHA standards are similar in content depending on the date the standard was finalized they have some notable differences. These standards:

- Establish exposure limits.
- Require “baseline sampling” to establish exposure limits.
- Require periodic monitoring based on the initial monitoring results. This can be quarterly, semi-annually, or annually.
- Establish criteria for the cessation of monitoring.

As an example of the requirements and differences, Table 4.1 contains language from two OSHA substance specific standards.

Compliance of monitoring is typically done over a small number of days to make a decision on compliance status and compliance activities that are defined by the exposure results and substance specific standards. These actions include

Table 4.1 Examples of OSHA monitoring requirements

OSHA Benzene Standard 29 CFR 1910.1028	OSHA Ethylene Oxide Standard 29 CFR 1910.1047
Determinations of employee exposure shall be made from breathing zone air samples that are representative of each employee's average exposure to airborne benzene	Determinations of employee exposure shall be made from breathing zone air samples that are representative of the 8-h TWA and 15-min short-term exposures of each employee
Representative 8-h TWA employee exposures shall be determined on the basis of one sample or samples representing the full shift exposure for each job classification in each work area	Representative 8-h TWA employee exposure shall be determined on the basis of one or more samples representing full-shift exposure for each shift for each job classification in each work area. Representative 15-min short-term employee exposures shall be determined on the basis of one or more samples representing 15-min exposures associated with operations that are most likely to produce exposures above the excursion limit for each shift for each job classification in each work area
Determinations of compliance with the STEL shall be made from 15 min employee breathing zone samples measured at operations where there is reason to believe exposures are high, such as where tanks are opened, filled, unloaded or gauged; where containers or process equipment are opened and where benzene is used for cleaning or as a solvent in an uncontrolled situation. The employer may use objective data, such as measurements from brief period measuring devices, to determine where STEL monitoring is needed	Representative 15-min short-term employee exposures shall be determined on the basis of one or more samples representing 15-min exposures associated with operations that are most likely to produce exposures above the excursion limit for each shift for each job classification in each work area
Except for initial monitoring as required under paragraph (e)(2) of this section, where the employer can document that one shift will consistently have higher employee exposures for an operation, the employer shall only be required to determine representative employee exposure for that operation during the shift on which the highest exposure is expected	Where the employer can document that exposure levels are equivalent for similar operations in different work shifts, the employer need only determine representative employee exposure for that operation during one shift
If the monitoring required by paragraph (e)(2)(i) of this section reveals employee exposure at or above the action level but at or below the TWA, the employer shall repeat such monitoring for each such employee at least every year	If the monitoring required by paragraph (d)(2) of this section reveals employee exposure at or above the action level but at or below the 8-h TWA, the employer shall repeat such monitoring for each such employee at least every 6 months
If the monitoring required by paragraph (e)(2)(i) of this section reveals employee exposure above the TWA, the employer shall repeat such monitoring for each such employee at least every six (6) months	If the monitoring required by paragraph (d)(2)(i) of this section reveals employee exposure above the 8-h TWA, the employer shall repeat such monitoring for each such employee at least every 3 months

Table 4.1 (continued)

OSHA Benzene Standard 29 CFR 1910.1028	OSHA Ethylene Oxide Standard 29 CFR 1910.1047
The employer may alter the monitoring schedule from every 6 months to annually for any employee for whom two consecutive measurements taken at least 7 days apart indicate that the employee exposure has decreased to the TWA or below, but is at or above the action level	The employer may alter the monitoring schedule from quarterly to semiannually for any employee for whom two consecutive measurements taken at least 7 days apart indicate that the employee's exposure has decreased to or below the 8-h TWA
If the periodic monitoring required by paragraph (e)(3) of this section reveals that employee exposures, as indicated by at least two consecutive measurements taken at least 7 days apart, are below the action level the employer may discontinue the monitoring for that employee, except as otherwise required by paragraph (e)(5)	If the initial monitoring required by paragraph (d)(2)(i) of this section reveals employee exposure to be below the action level, the employer may discontinue TWA monitoring for those employees whose exposures are represented by the initial monitoring

the designation of regulated areas, exposure controls needed including respiratory protection, training, medical surveillance and continued periodic exposure monitoring. This approach can often result in identifying the highest risk employees and evaluating worst case conditions. In other words there will be more monitoring results available for the highest exposed employees or employee groups. In general these results must be viewed with caution in terms of how they relate to evaluating exposures and health risk for a full worker population or classification of workers.

In summary compliance monitoring may well focus on the maximum risk employee to determine whether exposures are above or below established exposure limits. It has been recognized that data collected solely for the purpose of determining compliance usually reflects the exposures of the maximum risk employees within each exposure group. Such data can present risk assessors with a skewed or biased picture of the exposure experience of broader groups of employees under study.

Periodic Monitoring to Determine Ongoing Exposure Levels

This type of monitoring is often done to evaluate the representative exposure levels for a worker population or a group of employees considered to have similar exposure. You may see reports that speak to Similar Exposure Groups (SEGs). The assessment theory or strategy of SEGs is that for a group of employees who experience similar exposures to stressors, if one of the employees were monitored, the results of the monitoring can be used to predict the exposures of all the members of the group. This has become a common way to define occupational groups for the purposes of exposure evaluation and reporting. Various definitions can be found in publications and presentations but one commonly used is provided by (Mulhausen et al. 1998):

> A Similar Exposure Group (SEG) is a group of workers having the same general exposure profile for the agent(s) being studied because of the similarity and frequency of the tasks they perform, the materials and processes with which they work and the similarity of the way they perform those tasks.

This type of monitoring is more common in recent history and is considered good practice in developing a more comprehensive exposure assessment program. One benefit, or driver for periodic monitoring, is the ability to detect trends or persistent changes in measured exposures. This can be an effective way to evaluate the continued effectiveness of engineering or administrative controls in place to reduce employee exposures.

Response to Specific Complaints/Identified Problems

This may be another example of evaluating worst case exposures. In other words evaluating exposures in areas where alleged exposures have resulted in complaints or spills and releases have occurred may result in finding unusual circumstances such as use conditions causing high exposures that are not representative of routine or every day exposure profiles. These investigations are an important part of any workplace exposure evaluation program but the results must be taken in context as they relate to long term or routine exposure profiles.

Exposure Monitoring Techniques

In general quantitative monitoring data can be generated from (a) area samples, (b) personal inhalation samples, (c) dermal samples or (d) biological monitoring (Table 4.2).

a. Area samples are collected to represent the airborne concentration of a chemical in a specific location at a facility. Exposure is assessed in terms of ambient air concentrations in a given area over a specific time period. Measurement device can be of any size and power source needs are flexible and mobility or portability is not a requirement. The arrangement for area sampling can take many forms but is limited in utility depending on the workplace conditions and worker routines in the areas monitored.
   In work spaces, typically indoors, area samplers placed near employee work stations can provide consistent estimates of actual employee exposure potential depending on:
   - Time frame for area samples to be taken (e.g. every 15 min vs. every hour)
   - Knowledge of the consistency or nature of contaminant generation
   - Knowledge of employee work routines in these areas.

For example if the nature of the process or activity results in consistent contaminant release and there is detailed information on the time workers spent in that area a time weighted average exposure could be calculated or estimated.

Table 4.2 Important concepts for exposure sampling

a) Area samples are collected to represent the airborne concentration of a chemical in a specific location at a facility
b) Peak or ceiling samples are typically collected instantaneously through continuous monitoring or for 15 min or less. Short-term samples are collected over a designated period, typically less than 2 h. Full-shift samples are collected to represent a worker's inhalation exposure over an entire work shift and may be composed of a single sample or consecutive short-term samples
c) Dermal samples are collected to represent a worker's dermal exposure to a given chemical over a portion of the body which has been in contact with the chemical
d) Biological monitoring is defined by the American Industrial Hygiene Association Committee on Biological Monitoring as: "*The assessment of human exposure through the measurement of internal chemical markers of exposure, such as the chemical agent itself and/or one of its metabolites or an exposure related biochemical change unrelated or related to disease, in human biological samples*." There are a limited number of guidance values for chemicals measured in the body. The major sources of these values are published by the American Conference of Governmental Hygienist (ACGIH) and are known as biological exposure indices (BEIs). In addition to the 50 chemicals for which a BEI has been established, the American Industrial Hygiene Association (AIHA) has developed a Biological Environmental Exposure Level (BEEL) to more directly develop guidance values for chemicals which have the skin as their primary mode of exposure. Currently, one chemical, methylenedianiline (MDA), has an established BEEL

Area monitoring can be done through what are commonly known as fixed point continuous monitors. These instruments continuously monitor ambient conditions and provide results through visual or audible methods. These monitors can serve a valuable role in exposure assessment and control programs by virtue of being connected to visual and audible alarms, thus providing a means to reduce employee exposure to the detected high levels and providing trends analysis on equipment leaks or activities in the monitored areas that need attention to reduce contaminant generation.

b. Personal samples: this sampling technique is intended to represent a specific employee's exposure to airborne chemical or physical agents. In theory it is the most accurate representation of exposure that can be obtained. This is the primary exposure monitoring technique in use today. Personal sampling devices are attached to the workers and can provide a true, although time weighted average, estimate of exposure for whatever sampling period is chosen. These samples are collected to represent a worker's exposure during a specified time period; for example, short-term, and full-shift samples. Peak or ceiling samples may also be collected and are typically collected instantaneously through continuous monitoring or for 15 min or less. Short-term samples are collected over a designated period, typically less than 2 hours. Full-shift samples are collected to represent a worker's inhalation exposure over an entire work shift and may be composed of a single sample or consecutive short-term samples.
   In personal monitoring for chemical contaminants and some physical agents such as noise the measurement device is placed as close as possible to the employees breathing zone or receptor site such as the ear. This normally is accomplished

by attaching the collection media to the collar or lapel of the employee. This placement is dependent on the work clothing typically worn and whatever point of attachment is available and, as importantly, acceptable to the employee. Even with careful placement there are multiple variables that can impact the ability of the sampling results to accurately represent inhalation exposures. The nature of the work and contaminant generation relative to the side of the employee that the monitor is placed can impact sample collection. It has been demonstrated that left and right handed employees can create different exposure profiles for the same activity. In a similar vein the nature of the work and whether the employee is left or right handed can impact proximity to the agent being sampled.

Worker acceptance of personal monitoring has created significant challenges in the past. Early methods for personal sampling relied on what were then considered portable, battery powered devices that employees were asked to wear throughout the designed sampling period. Early sampling devices were often heavy and in some cases quite loud. As a result employees were known to dislike these intrusions into their normal work and work "break" routines. Industrial Hygienist and other sampling personnel spent a lot of time monitoring the locations of their sampling devices during sampling campaigns and in the end, how representative the samples were could be in question.

With the advent of passive air sampling techniques not requiring an electric pump these conditions improved. It can be an important factor to understand the specific methodologies utilized to generate personal sampling data to be used in a risk assessment. Trends in exposure levels, up or down, could be related to the changing technologies and subsequent worker acceptance of the sampling methodology in use.

c. Dermal samples: these samples are collected to represent a worker's dermal exposure to a given chemical over a portion of the body which has been in contact with the chemical. The dermal component of personal exposure is an often unevaluated or under evaluated component of exposure assessments for several reasons. These include:

   - Difficulty in obtaining quantitative sampling for potential and real skin exposures
   - Lack of a driver such as compliance sampling for air mediated permissible exposure limits normally based on an inhalation or other exposure basis
   - A limited number of chemical agents designated as "skin hazards"

Dermal exposure can be evaluated with various wipe or swab samples from employee workplaces and employee skin. However these protocols and techniques are generally not well defined and are not commonly used.

The US OSHA Technical manual does address assessing dermal exposures in several ways. The following excerpt is taken from published OSHA guidance: (OSHA 2014)

> Skin wipe samples taken on potentially exposed areas of an employee's body are a useful technique for demonstrating exposure to a recognized hazard. For water-soluble chemicals, a wipe pad moistened with deionized water can be used to wipe the skin. Generally, the best procedure is to allow employees to use the wipe pad to clean their skin surfaces, and then have them insert the wipe pad into a clean container, which is labeled and sealed. Hands,

forearms, faces, and possibly feet may be exposed to contaminants that a wipe sample of the skin can be used to establish exposure. Include a blank water sample and use only deionized water, or another source of water approved by the laboratory, for analysis purposes.

d. Biological monitoring may also be used to determine an employee's overall exposure to a given chemical by measuring the appropriate determinant in biological specimens collected from exposed workers at the specified time. While biological monitoring provides information complementary to air monitoring, interpretation of data can be difficult due to variability in the physiological and health status of the individual, exposure sources, individual life style, analytical errors, etc. If biological monitoring data are available, this fact should be noted in the exposure assessment.

There are a limited number of guidance values for chemicals measured in the body. The major sources of these values are published by the ACGIH and are known as biological exposure indices or BEIs, There are 50 chemicals for which a BEI has been established (ACGIH 2013; OSHA 2014).

This section illustrates that the risk assessor must use caution when utilizing historical exposure data for risk assessment purposes. The purpose of the monitoring and methods used can result in a wide variability of reported exposures. Much of the historical data is generated for compliance purposes. That meaning worst case exposures are estimated and measured to compare to regulatory or other exposure limits for singular or only a few chemicals. This data may not represent the average or typical exposure for any given group of workers. Seldom is the total worker exposure experience evaluated or reported. Actual exposures and therefore risk can easily be overestimated.

Air Sampling and Analysis for Occupational Exposures to Gases and Vapors (Conrad and Soule 1998; Peach and Carr 1986; Huey 1996; Hahne 1996; Dietrich 1998)

Two general types of sampling instrumentation are employed to measure worker exposure gas and vapors. Those are:

- Methods that require laboratory analysis of collected samples and:
- Direct reading instrumentation/methods that provide a near immediate analysis of the atmosphere evaluated

Methods requiring analysis

In general there are two categories of air samples taken for further analysis:

- Grab or short term samples
- Long term or time weighted average samples, sometimes referred to as integrated samples

a. Grab Samples: these samples are collected over a short period of time, typically for less than 15 min. The purpose of the sampling and knowledge of workplace conditions and activities will drive the actual time used for the sampling. Results from grab samples are indicative of contaminant levels at the sampling location at a specific point in time. Grab sampling is useful in characterizing different phases of a cyclic process or specific tasks and activities. Where it is known that no exposure exists in between the times monitored a cumulative exposure or time weighted average can then be calculated for the individual monitored. Over time there has been a wide range devices used to collect grab samples. These include:

   - Evacuated flasks
   - Syringes with a known volume
   - Plastic bags, and
   - Liquid displacement containers

b. Long term samples: these samples are taken over a longer period of time with a known or measured volume of air. Depending on the workplace and activities being measured the sampling period may vary from greater than 15 min to a 8 hours. It should be noted that 8 hours is a common time period for this type of sampling based on the fact that occupational exposure limits and compliance requirements are often based on 8 hours time weighted averages. Long term sampling is often employed:

   - when worker exposure potential is known to be variable over any given time during the workday
   - when the workers are known to be mobile during the work shift and undertaking activity in a unknown on non routine manner
   - to obtain a more reliable time weighted average estimate of exposure over a full work shift
   - when anticipated exposure levels are low and a large sample volume is needed based the detection limits or sensitivity limitations of the analytical method to be used

The collection of long term samples typically involves the extraction of the contaminant from a sampled air stream using the principles of absorption, adsorption, or less commonly condensation.

Absorption: in this method the contaminant is collected from the air stream and concentrated in a solution by pulling through an absorbing liquid or through a reaction with an absorbing reagent. There are various types of absorbing devices with a range of effectiveness depending on the known contaminate and possible concentrations. These include simple gas wash bottles (impingers), fritted bubblers and glass beaded columns. The selection of the appropriate device is impacted by the solubility and reactivity of the contaminant being collected.

Adsorption: This refers to the collection or adherence of sampled gases and vapors to the surface of a solid sorbent without physical or chemical changes occurring. This is the most common methodology used in exposure measurements. It is used for collection of most insoluble and non reactive gases and vapors in

the workplace. Multiple solid sorbents are utilized depending on the chemical agents being collected. Sorbents are chosen for high surface areas and affinity for organic molecules. Sorbents commonly used are activated charcoal, silica gel and porous polymers.

The sampled air is drawn across a small tube packed with the appropriate sorbent material. As the air passes across the sorbent the contaminant molecules adsorb to the surface of the sorbent. The contaminant is then desorbed by a liquid solvent or thermally (high heat) for subsequent analysis. Solvent desorption was the standard method for many years but thermal desorption has gained utility and popularity based on the increases in accuracy and detection limits found with less losses of contaminate when compared to the liquid desorption method.

Another adsorption sampling method that became commercially available in the late seventies is the passive dosimeter. The process of passive dosimetry is based on Fick's first law of diffusion. The concentration of the contaminant is passed to a solid sorbent bed through a diffusion layer, or barrier that controls the sampling rate of the device. Passive dosimeters have improved exposure measurements through the elimination of sampling pump errors, worker acceptability and general ease of use.

Direct Reading Instrumentation

Direct reading instruments are an important tool for exposure evaluation. These instruments allow for real time or nearly instantaneous measurement of contaminant concentrations in the work environment. These instruments eliminate the need to wait for analytical results of measurements and are generally used to obtain short term exposure evaluations or to evaluate the potential for exposures to occur therefore allow for control measures to be taken to prevent exposures. An example of this would be for measurements to be taken before entry into a confined space or other enclosed work area.

Some direct reading instruments are designed to take longer term samples through data logging capabilities and by continuous sampling through an air pump. The utility of these samples to represent time weighted average exposures is impacted by the detection limits of the method in use and the general accuracy and precision of the results.

Direct reading instruments come in many sizes and degrees of complexity. With recent advances in instrument design they can be small enough to effectively serve as a personal monitor. They are generally available for gases and vapors and aerosols or particulates.

For the purposes of this chapter direct reading instrumentation can be put in two basic groups.

- Those that produce a color change either in solution or detector tubes through which the air sample has been drawn. The degree of color change or the length of stain found indicates concentration.
- Those that have electronic circuitry for measuring the concentration and displaying the results on a dial or digitally.

a. Colorimetric Indicators: Common indicator systems in this category are liquid reagents and detector (indicator) tubes. They utilize the chemical properties of specific atmospheric contaminants to produce a reaction with a color-producing reagent. This type of system generally provides semi quantitative results of contaminant concentrations. The accuracy, within their limits of detection, depends greatly on the skill and experience of the operator and knowledge of the atmosphere being sampled. These methods, if done well, can provide a relatively quick and inexpensive estimate of the concentration of the contaminant of interest.

   Of the systems above the "detector tube" is the most common in part due to their ease of use, fast results, and a large number of substances that can be sampled. These devices have been used for many years in a wide range of applications. A detector tube is a hermetically sealed glass tube containing an inert solid granular material. This material is impregnated with one or more reagents that change color when reacting with specific chemicals or groups of chemicals. The ends of the tube are broken and a known volume of air is pulled through the tube. The length of the resulting color change, or the intensity of the color change, is compared to reference information provided by the manufacturer to obtain an airborne concentration. The volume pulled through the tube can be controlled through various mechanisms. Results are usually obtained in a matter of minutes. Selection of the detector tube to use is determined by the anticipated contaminates present and the concentration range to be sampled.

   Detector tubes are limited in utility by multiple factors including accuracy limits typically in the ±25 % range, temperatures, humidity, atmospheric pressures, shelf life, interfering chemicals and user care and attention to manufacturer's directions for sampling. Even with these limitations they have proven to be reliable and practical devices for getting a semi quantitative evaluation of contaminant levels in the work environment.

b. Electronic Direct Reading Instrumentation: this type of direct reading instrument incorporates electronic sensors utilizing a range of technologies to quickly measure single gases or vapors (i.e. oxygen and carbon monoxide) or multiple gases and vapors of different chemicals. These instruments are most useful for quantifying levels of exposure to a wide range of hydrocarbons in the workplace. Details on some of the more common instrument sensor technologies are discussed below.

Infrared (IR) Spectrometers: These instruments measure the IR absorption spectrum of the chemicals being sampled. Nearly all molecules absorb IR radiation at specific wavelengths and can be identified in this manner. IR instruments are relatively simple to operate and can be dedicated to one chemical or used to detect multiple chemicals. These instruments can be used to quantify a wide variety of specific chemicals at concentrations as low as 1–10 ppm. IR instruments can be used to identify unknown chemicals but multiple chemicals may register on the instrument in a similar fashion creating interferences. These instruments are more commonly used to measure the level of known contaminants. These instruments can be relatively expensive and somewhat bulky to use.

Photoionization detectors (PID): In PIDs the gas sample is ionized by an ultraviolet lamp. The number of ions formed and the strength of the electrical signal produced are directly proportional to mass and concentration. These instruments are generally non specific and provide qualitative information on the amount and class of chemicals present. PIDs are calibrated against a specific gas, such as isobutylene, and all readings are in equivalent units. PIDs are relatively inexpensive lightweight, and require little power. PIDs may experience interferences from high humidity, particulates, and hot or corrosive atmospheres.

Flame Ionization Detectors (FID): FIDs are essentially carbon counters. The gas sample is pyrolized in a hydrogen flame to produce carbon ions. The resulting electrical signal indicates the amount of carbon present. The instrument is calibrated against specific known gas samples and the readings are in equivalents units. Some FIDs are coupled with a gas chromatograph to allow for quantification of specific compounds. These instruments can be relatively expensive and somewhat bulky to use. In field use the flames are also prone to being extinguished by air currents ("blow out".)

This section illustrates how many choices must be made in the sampling and analytical methodology to be used when generating exposure assessment measurements. These choices have a direct impact on the variability and reliability of the data reported.

Air Sampling and Analysis for Occupational Exposures to Particulates (Todd 1998; Johnson and Swift 1998)

It is important to understand the terminology and definitions used to define "particulates" in the occupational exposure setting. There are wide ranges of particulate materials involved in industrial process and the generation of particulates of concern can come from multiple physical and chemical actions. Following are common definitions (Table 4.3):

Table 4.3 Definitions for dust/particles

Dusts	Particles rendered airborne during crushing or grinding of rock like material
Fumes	Airborne solid particles formed above molten metal
Mists	Droplets rendered airborne by bubbling, boiling, spraying or splashing
Smokes	Particles resulting from incomplete combustion of organic matter
Fibers	Elongated particles typically with an aspect ratio of greater than 3:1
Aerosol	A grouping of solid or liquid particles dispersed in air

Airborne particulates can affect the skin but inhaled particles are normally the primary occupational exposure concern. This section will focus on the inhaled hazard. The hazard potential and sampling needs for particulates are determined principally by chemical composition of the particulate of concern, mass concentration measured in the environment and size characteristics of the particulate of concern.

To assess the possible health effects of airborne particulate matter, exposure standards and guidelines have been issued for different sizes of particles. Historically the basic distinction in size has been between "total dust" and "respirable" dust.

- Respirable Dust—dust particles that are less than 10 micron in size and can penetrate into the deepest parts of the lungs.
- Total Dust—dust particles that are greater than 10 micron in size and get caught up in the upper respiratory tract and are often taken care of by coughing and sneezing.

Results of quantitative assessments for much of the dust sampling that has been done since 1970 will be expressed in terms of these size classifications. More recent particle descriptions and subsequent sampling results may include reporting on three fractions or sizes of particulate: inhalable fraction (< 100 μm AED*)– can be breathed into nose or mouth; thoracic fraction (< 25 μm AED)—can penetrate head airways and enter lung airways; and respirable fraction (< 10 μm AED)—can penetrate beyond terminal bronchioles to gas exchange region.

**AED: The Aerodynamic Equivalent Diameter (AED) of a particle is the diameter of a unit density sphere that would have the identical settling velocity as the particle. It is a measure of behavior of particle in air and a function of particle diameter, density, shape, and surface characteristics. The AED determines site of deposition in lung and impacts air sampling characteristics. AED is referenced to spherical drop of water with identical settling velocity* (Todd 1998; Johnson and Swift 1998).

This particle size distinction also required new sampling technology. Multiple sampling devices are now available that provide selective particle size sampling to compare to these three fractions of particulate. These devices began to be used in the late 1980s.

Methods Requiring Analysis

Air sampling for dusts is similar to gas and vapor sampling in that sampling is usually done by drawing a measured volume of air through a filter to collect the airborne contaminate of concern. The filter is pre weighed and the collected dust is impinged on the filter for a gravimetric determination of the mass collected. This mass is then converted to a concentration number. Older methods relied on particle counting to produce a concentration metric. Common filter materials include mixed cellulose ester, polyvinyl chloride (PVC), glass fiber/quartz and Teflon. The choice of the media can be dependent on the pore size that is appropriate for the particulate and the degree of chemical analysis that may be needed on the sample.

Respirable Dust

Collected onto a filter or other collection device of a type and pore size that is appropriate for the particulate being sampled (typically PVC filters). Preceding the filter, however, is a particle size-selective device, typically a cyclone, that will separate the respirable fraction from the non-respirable fraction when connected to a pump sampling at the designated flow rate.

Total Dust

Dust that is captured onto a 37-mm filter loaded into a cassette and connected to a sampling pump calibrated to a flow of at least 1 L/min. The filter should be of a type and pore size appropriate to the particulate being sampled. Samples are collected in an area or in the breathing zone of workers.

Direct Reading Particulate Monitoring

There are increasing numbers of direct-reading dust monitors on the market today that when properly calibrated provide an accurate measure of airborne respirable dust. These are mainly based on light scattering technology where a light source (usually produced by a laser or diode) is collimated and illuminates dust entering the sensing volume. The intensity of the light scattered at a particular angle is proportional to the dust concentration. The monitors are usually calibrated in the factory using a 'standard' test dust and are adjusted to agree with respirable dust concentration measurements made using reference methods. In the field monitors can be exposed to a wide range of dusts with differing physical properties such as particle size, refractive index and particle shape, which will affect their response by varying degrees.

Exposure Assessment Variability

Regardless of the reason for conducting occupational exposure assessments the objective is to accurately assess employee exposures. The use of statistical methods in the exposure assessment process is important and necessary because of these recognized causes of variation. There are many literature sources that describe a wide range of statistical concepts and practical tools such as spreadsheet programs and smart phone applications to aid Industrial Hygienists and others in designing survey strategy and interpreting sampling results. These principles and applications are beyond the scope of this chapter.

Even in statistically-selected, well-done studies, there may be high variability in the characterization of worker exposure. Measurements at a plant made over a period of no more than a few days may be all that are available to characterize exposures over an entire year or a period of years. Seasonal variability, interday and intraday variability, and changes in the process or worker activities can cause the exposure to vary from that measured on a single day. Temperature changes can affect evaporation rates, and seasonal changes in natural ventilation affect exposure. Sampling methods and time periods can also vary. Seldom can all these variables be measured and accounted for (OPPT, USEPA 1994).

Regardless of the care taken in study design and conduct there are known causes of variation and varying degrees of impact on the accuracy and precision of the data generated. It is important to recognize these potential variables when considering the utility of the data available. Care must be taken not to assume there are precise lines of demarcation between a reported exposures or exposure averages of for example 1.0 vs. 2.0 ppm.

Following is a discussion of the more common recognized sources of errors and variation that impact estimates of occupational exposure (Table 4.4).

In the list items 1–4 are sometimes called statistical errors and can be accounted for or managed, but not prevented, by statistical analysis. This is to some degree assuming a reasonable number of samples for the target individual or worker population is available. Systematic errors under number 5 include both instrumental errors and, as noted by Leidel, Busch and Lynch, "goofs or blunders of the fallible human using the equipment" (Leidel et al. 1977).

Random errors included in 1 and 2 are often quantified and their effects minimized by the application of statistically based quality control programs. For example for most published and validated sampling and analytical methods the typical variation is known.

Table 4.4 Sources of sampling errors and variability

1.	Random Sampling device errors such as fluctuations in pump flow rate
2.	Random analytical method errors such as deviations in procedures used by individual analyst
3.	Random intraday (within a day or sampling time) environmental fluctuations in contaminant concentrations
4.	Random interday (between days) environmental fluctuations in contaminant concentrations
5.	Systematic errors in the measurement process such as calibration technique, erroneous recording of data or typos, etc.
6.	Systematic changes in a contaminant's concentration due to: (a) employee movement to different work areas during a working day; (b) change in individual employee habits or methods for accomplishing specific task resulting in exposure potential; (c) seasonal variations caused by door or window opening, and (d) changes in the efficiency of control devices such as exhaust fans

Random environmental fluctuations in 3 and 4 are well known to be influenced by the specific processes that generate the chemical of concern and the work habits or routines of the employee. It should be noted that the random environmental fluctuations of a contaminant in a process or plant may greatly exceed the random variation in concentrations caused sampling and analytical procedures. This can be by factor of 10–20.

Systematic errors in 5 and 6 are simply a fact of the overall exposure assessment process and cannot be controlled or accounted for with statistical methods. As sampling technology and calibration techniques have improved over the years these factors have been minimized. The factors associated with human behavior, such as following operating procedures in the same way as other employees emphasize the importance of observation and employee interviews in the overall assessment process.

Summary

Risk assessments are impacted by the quality and derivation of the exposure assessment data that is available. In general risk assessors will have to work with historical data generated over periods in time when the assessment strategies changed, technology for creating quantitative data rapidly evolved and the ability to access and analyze the data available evolved from hard copy files to sophisticated databases tied to other occupational elements such as medical surveillance records.

Variability in the exposure assessment process can occur as a result of the sampling strategy, sampling and analytical methods utilized, variability inherent in workplace activities and environments and in the end human error in part of the process.

Therefore it is important to have a working knowledge of how exposure assessment data has been and is now generated, the rationale used for determining assessment strategy and practices, the technologies used to gather data and information and the limitations, or strengths and weaknesses of the data a risk assessor will have to work with. Enlisting the assistance of an exposure assessment professional such as an experienced Industrial Hygienist can help bring value and precision to the historical data available for use in any risk assessment effort.

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Chapter 5
Risk Characterization for Human Health Risk Assessments

Rhian B. Cope

Abstract The objectives of this chapter are to provide an introduction to the basics of risk characterization. The areas of risk characterization covered in this chapter include: an introduction to the role of risk characterization in the risk assessment process, the overall holistic purposes of a good risk characterization, principles of transparency, clarity, consistency and reasonableness, how a risk characterization is used, and introduction to some of the metrics that may be used in a risk characterization, the relationships between sensitivity, specificity and predictive values, Reasons's accident causation model, common factors that drive decision-makers and an introduction to Graham's hierarchy of disagreement. Overall, the chapter provides a systematic and stepwise process for developing a risk characterization. This chapter is the private opinion of Dr R. B. Cope and does not reflect any official policy or legal position of the Government of Australia or any of its departments or agencies.

Keywords Transparency · Clarity · Consistency · Reasonableness · Graham's hierarchy of disagreement · Reasons's accident causation model

Student Learning Objectives

- Understand the role of risk characterization in the risk assessment process
- Understand the overall holistic purposes of a good risk characterization
- Understand the principles of transparency, clarity, consistency and reasonableness
- Understand how a risk characterization is used
- Understand the various metrics that may be used in a risk characterization
- Understand the relationships between sensitivity, specificity and predictive values
- Be aware of Reasons's accident causation model
- Understand the stepwise process involved in developing a risk characterization
- Be aware of the common factors that drive decision-makers
- Understand Graham's hierarchy of disagreement

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Introduction and Philosophical Underpinnings

Risk characterization refers to the: *"… synthesis and summary of information about a potentially hazardous situation that addresses the needs and interests of decision makers and of interested and affected parties. Risk characterization is a prelude to decision making and depends on an iterative, analytic-deliberative process." In critical practical terms, risk characterization is "the process of organizing, evaluating and communicating information about the nature, strength of evidence and the likelihood of adverse health or ecological effects from particular exposures* (CRARM 1997)."

Within the above context, it is essential to realize that in a human health risk assessment, the risk characterization process fits into a 5 step process (Fowle and Dearfield 2000):

- Hazard identification: the identification of the causal relationship between a particular chemical or substance and the potential to cause particular health effects (i.e. harm or hazard).
- Dose response assessment: identification of the relationship between the level of exposure by a particular route or routes and the risk of a particular hazard. This involves the derivation of toxicological thresholds such as reference doses, acceptable daily intakes, derived no effect levels, cancer slope factors, thresholds of toxicological concern and so forth.
- Exposure assessment: measurement (either directly or by modeling) of the level of human exposure (by all relevant routes) to a particular chemical or substance.
- Risk characterization: a description of the type(s) and magnitudes of human risks, including the associated uncertainties of the analysis. This may include an effort to codify the severity of the risk e.g. cancer or death are potentially more severe risks than other biological effects.
- Risk communication: a process of interactive exchange or information and opinion between risk assessors, risk managers and relevant stakeholders. This process often has critical implications for the process of risk characterization because of the phenomenon of perceived rather than actual risk.

The above processes are not always linear or unidirectional in nature. In reality, the different steps often operate concurrently and with one phase feeding back into all the others. It is important to note that within human health risk assessment, separate risk characterizations are associated with each of the steps in the process (Fowle and Dearfield 2000). These "component characterizations carry forward the key findings, assumptions, strengths and limitations, etc. for each section and provide a fundamental set of information used in an integrative analysis that must be conveyed in the final overall risk characterization."

The critical holistic purposes of a final overall risk characterization is to convey to the relevant stakeholders *why the risk assessors assessed the risk the way that they did*, with clear and exact descriptions of: (a) the relevant context and frame of

reference of the analyses; (b) the available data (and, critically, its limitations and short-comings, and any critical data gaps); (c) why the analyses were carried out the way that it was; (d) the uncertainties and limitations of the analyses; (e) potential alternate analyses; (f) the choices made during the analyses. The word "clear" is critical. It is important to realize that when trying to communicate risk assessments and risk characterizations to stakeholders *that* "*any perception of deception*" *is fatal to the process,* irrespective of the scientific quality and integrity of the work:

> "A good risk characterization will restate the scope of the assessment, express results clearly, articulate major assumptions and uncertainties, identify reasonable alternative interpretations, and separate scientific conclusions from policy judgments. The Risk Characterization Policy calls for the explanation of the choices made to be highly visible (Fowle and Dearfield 2000)"

Furthermore it is also important to realize that risk characterization is not always about the science: "a good risk characterization is also about making it clear that science doesn't tell us certain things and that policy [i.e. political] choices must be made (Fowle and Dearfield 2000)."

Risk characterizations are *inherently dependent upon context* and *frame of reference*. If the context and or frame of reference change, then the risk characterization may no longer be valid. Furthermore, the process of human health risk assessment operates as a science i.e. risk assessors operate within the context of the *reliable* body of knowledge (i.e. science). The risk assessor, as scientist, uses a systematic approach to build and organize knowledge in the form of *testable* explanations and predictions (i.e. hypotheses). As with any form of science, human health risk assessment operates using the principles of critical rationalism and falsifiability (Popper 2002). Critical rationalism requires that scientific theories and any other claims to knowledge should be rationally criticized, and should be subjected to tests that may falsify them. Falsifiability (refutability) is the inherent possibility that a scientific hypothesis may prove to be false if it is possible to conceive an observation, a test or an argument that demonstrates that the stated scientific position is incorrect. Based on this scientific system, there are three possible outcomes to any scientific hypothesis: correct, incorrect and not even wrong (i.e. meaningless). Of these outcomes proving a hypothesis correct or incorrect provides valuable new information. "Not even wrong" results provide no advancement of knowledge: such results are meaningless.

Like everything else in human health risk assessment (and science in general), risk characterizations will change as knowledge develops and relevant scientific paradigm shifts ensue. Risk characterizations are not a static, fixed entities (Eliot 1943):

> "For last year's words belong to last year's language
> And next year's words await another voice."

As with any other scientific activity, risk characterization requires adequate independent peer review and peer evaluation.

The TCCR Principles of Risk Characterization

The fundamental principles of risk characterization are (Fowle and Dearfield 2000):

- **T**ransparency.
- **C**larity.
- **C**onsistency.
- **R**easonableness.

While TCCR principles are critical for risk characterization, it is also apparent that a lack of TCCR principles in other stages of the risk assessment process will inevitably "filter through" to the risk characterization process. Furthermore, attempts to compensate for TCCR deficiencies in the other components of a risk assessment will only feed the "perception of deception" amongst the consumers of the risk assessment process. In point of fact, *it is specifically not the role of the risk characterization process to mask the limitations, uncertainties and deficiencies of the other components of a human health risk assessment*. *A good risk characterization will, in fact, do the diametric opposite of this*. *A good risk characterization will point the way for how the limitations, uncertainties, deficiencies and critical data gaps can be overcome and how this will improve the overall quality of the risk assessment!* This may involve gathering additional information, performing new tests or gaining a better understanding of modes of action. Furthermore, like most analytical processes, risk characterizations are only as good as the data that supports them and are susceptible to the "garbage in, garbage out" phenomenon. Accordingly, there are established criteria for each of the TCCR principles.

Transparency Criteria The objective of transparency is explicitness and full disclosure. Transparency is the keystone of the TCCR principles because, when followed, this principle leads to clarity, consistency and reasonableness (Fowle and Dearfield 2000). Transparency also requires an understanding of the language of the audience and a tailoring of the writing to this specific audience. Transparency requires, at a minimum, disclosure of the following: (a) the assessment approach employed; (b) the use of assumptions and extrapolations and their impact on the assessment; (c) the use of models vs. measurements and their impact on the assessment; (d) the plausible alternatives and the choices made among those alternatives. This includes the impact of one choice versus another on the assessment; (e) significant data gaps and their implications for the assessment; (f) scientific conclusions identified separately from default assumptions and policy decisions; (g) the major risk conclusions and the assessor's confidence and uncertainties in them; and (h) the relative strength of each risk assessment component and its impact on the overall assessment (e.g., the case for the agent posing a hazard is strong, but the overall assessment of risk is weak because the case for exposure is weak).

Clarity Criteria The objective of "clarity" is to make the risk assessment process free from obscurity and easy to understand by all stakeholders (including those who are not scientists!). Again, understanding the language of the audience and a tailoring of the writing to this specific audience are essential components. Clarity involves (Fowle and Dearfield 2000): (a) brevity (although not to the extreme of

providing oversimplified "sound bites"); (b) avoiding jargon and technical terms. If they must be used, then they should be clearly explained; (c) using plain language; (d) providing clear quantitative estimations of risk; (e) addressing different communication styles by the use of tables and graphics; (f) avoiding complexity where it is not necessary a component of conveying the message; (g) using clear and appropriate equations to efficiently display mathematical relationships (complex equations should be footnoted or referred to in the technical risk assessment); (h) not expecting the audience to "read between the lines" or understand inherent, but unstated, conclusions. In other words, even the obvious needs to be clearly stated.

Criteria for Consistency Consistency helps the reader form a context regarding the material. Most commonly, consistency refers to the presentation of the material in a risk assessment. Different regulatory agencies and organizations have different basic formats for presenting a risk assessment. However, consistency does not mean blind observance and the stifling of innovation. Consistency may involve: (a) following statutory/legislative requirements or precedents; (b) following international consensus; (c) placing the current risk assessment in context with other similar evaluations; (d) defining and explaining the purpose of the risk assessment in terms of regulatory action, policy analysis or priority setting; (e) defining the level of effort e.g. a screening assessment versus an in-depth analysis.

Criteria for Reasonableness Reasonableness refers to the consistency of the findings of the risk assessment within the context of the relevant science. While it is entirely possible that a new risk assessment may indeed discover something entirely new to science, triggering a paradigm shift, it is more likely that the risk assessment will result in an incremental increase in the body of reliable knowledge and understanding. Risk assessments are rarely performed in complete isolation from existing knowledge. Thus a well develop human health risk assessments will display biological plausibility, an overtly logical path, general common sense and demonstrable good scientific judgment. Reasonableness is achieved when (Fowle and Dearfield 2000): (a) the risk characterization is determined to be sound by the scientific community (i.e. peer review) and the components of the risk characterization are well integrated into an overall conclusion of risk which is complete, informative, well balanced and useful for decision making; (b) the characterization is based on the best available scientific information; (c) the assessment uses generally accepted and reliable scientific knowledge e.g. the critical data was not published in the Journal of Obscurity and never replicated under reliable circumstances; (d) appropriate plausible alternative estimates of risk under various candidate risk management alternatives are identified and explained.

How is Risk Characterization Used?

A high quality risk characterization will communicate "the key strengths and weakness of an [risk] assessment through a conscious and deliberate effort to bring all the important considerations about risk into an integrated picture (Fowle and Dearfield 2000)."

In simplistic terms, a good risk characterization will make the key elements of the story *blindingly obvious* and easier to communicate. Hopefully, this will make the risk assessment easier to explain, justify and defend within the less than scientific processes that operate within regulatory and political systems. Fundamentally, these aspects usually lead to better and more informed decisions, more realistic risk perception, trust, and credibility. *Without trust, credibility and realistic perceptions of risk, no risk assessment will ever be accepted, let alone acted upon irrespective of its technical and scientific quality*.

Risk Attributes and Metrics for Risk Characterization

In addition to a descriptive component, many risk characterizations will use semi-quantitative to quantitative estimates of risk (or excess risk over background). It is critical to remember that such estimates always carry with them a degree of uncertainty and error. Numerical methods for risk characterization are often over-interpreted in terms of their accuracy. Within this context, it is should be remembered that when any two measurements are divided, the associated error and variance for each variable in the equation is *multiplied*. For example, if a risk ratio is calculated by dividing an exposure estimate by a toxicological threshold, and if both the exposure estimate and the toxicological threshold have associated variances of 5%, the overall variance for the risk ratio will be $5 \times 5 = 25\%$. When variables are added together, the associated error and variance for each variable are also added. For example, if you take the case of adding two component risk estimates each with an associated error of 5 in order to calculate an overall risk, the error associated with the overall risk will be $5 + 5\% = 10\%$. For these reasons, it is important not to over-interpret or over-represent the accuracy of results of these calculations. In particular, care should be taken with the number of decimal places used as this has direct implications on the accuracy that is implied. For example a risk ratio listed as 1 implies that the error associated with this number is ± 0.5. However, a risk ratio listed as 1.0 implies that the error associated with this number is ± 0.1.

The risk attribute "exposed population" is defined by two metrics: population size and the characteristics of the affected population. Within this context, population size refers to the number of individuals within a population who could be adversely affected within a specified timeframe. The metric of affected population is designed to capture sub-populations of particular concern (e.g. hyper-susceptible groups, groups with higher levels of exposure) such as children, the elderly, particular genetic or cultural or ethnic or socioeconomic groups, people with pre-existing disease, pregnant women and the immunocompromised. All the subsequent risk attributes and metrics discussed below, either explicitly or implicitly, take into account the risk attribute of the exposed population and its associated metrics of population size and population characteristics.

Hazard quotients (HQs) or risk quotients (RQs) are commonly used in toxicology-related human health risk assessments for non-cancer endpoints. Confusingly,

various regulatory authorities use different names and different terminologies for what is effectively the same concept. A HQ or RQ is simply a ratio of exposure to a specified toxicological threshold i.e.

$$RQ = \frac{Exposure}{Toxicological\ Dose\ Threshold}$$

The fact that the equation incorporates the concept of a dose threshold explicitly assumes that the toxicological effect is, in fact, a threshold response. RQ values of this type are *not appropriate for non-threshold toxicological responses such as mutagenesis and classical mutagenic carcinogenesis* (although they are commonly misused for this purpose). Various toxicological dose thresholds are used such as reference doses (RfDs), reference concentrations (RfCs), acceptable operator exposure levels (AOELs), derived no effect levels (DNELs), acceptable daily intakes (ADIs), non-cancer threshold of toxicological concern (TTC) etc. An RQ of ≤ 1 indicates that the risks of adverse non-cancer effects are low and is often defined as being of negligible risk. It is critical to note the following:

- Toxicological thresholds such as RfDs, AOELs, DNELs, ADIs (TTC) etc. and so forth already incorporate factors that (hopefully), take into account the uncertainty of these values.
- Toxicological thresholds such as RfDs, AOELs, DNELs, ADIs etc.by definition, are supposed to take into account sensitive subgroups of a population (and hence the attributes and metrics of the exposed population).
- Toxicological thresholds such as RfDs, AOELs, DNELs, and ADIs etc. by definition have an uncertainty spanning up to an order of magnitude. Given that the RQ calculation is one of division, this will *multiply* the uncertainties associated with both the toxicological threshold and the exposure assessment. *Thus it is particularly important not to over represent and/or over interpret the accuracy of RQ values*.
- RQ values *are not statistical probabilities of harm* occurring (i.e. mathematical estimates of risk). They are, in fact, more akin to a semi-quantitative indicator. Notably the level of concern does not increase in a linear manner as the RQ increases. In other words, as the RQ increases above 1, there is certainly an indication of an increased risk of an adverse effect, *but we do not know by how much* i.e. an RQ of 100 *does not* indicate a risk that is ten times higher than an RQ of 10.
- RQ values cannot be used to compare across different chemicals or different evaluations because toxicological thresholds do not generally have the same level of accuracy or precision and are generally not based on the same severity of effect across toxicological evaluations.
- RQs are often specific for a given duration of exposure i.e. acute, sub-chronic or chronic. The reason for this is that both the exposure data and toxicological thresholds from which RQ values are derived are also usually time specific.

RQs may only be available for individual compounds in a mixture. Under these circumstances the individual RQ values for each component of a chemical mixture

are assumed (unless there is specific data to contradict this assumption) to act additively. Thus a risk index (RI) can be calculated:

$$RI = RQ_1 + RQ_2 + RQ_3 + \cdots$$

This screening level approach is usually regarded as sufficient provided that the RI is ≤a predefined decision criterion. If the RI is >than the decision criterion, then a more refined analysis is required.

Notably, the RI screening approach assumes nothing about the toxicological modes of action and exposures (except that their effects are somehow additive) and the target organs/systems affected. This involves a series of massive, although unstated, assumptions; not the least of which is the assumption of dose additivity. More refined approaches will often involve subgrouping mixture components by toxicological similarity (e.g. similar target organ, or similar effect or similar mode(s) of action). This technique allows for the calculation of a target organ-specific risk quotient for particular subgroups (EPA 1986, 2000).

A margin of exposure (MoE) is the ratio of a NOAEL or LOAEL to an exposure level. MoEs have been classically used for pesticides. The US EPA regards a MoE of ≥100 for a NOAEL derived value and ≥1000 for a LOAEL derived value as representing a low level of concern. Recently the European Food Safety Authority (EFSA) has applied the MoE methodology too classically mutagenic (i.e. agents that directly interact with DNA) carcinogens. The EFSA MoE method is the ratio between the benchmark dose low 10% and the level of exposure. Under the EFSA system MoEs of ≥10,000 imply a low level of concern from a public health point of view.

For cancer endpoints occurring via classical direct DNA mutagenic modes of action (i.e. directly interact with DNA to produce DNA or chromosomal damage), an actual risk calculation is performed:

$$Excess\,Cancer\,Risk = E_L \times cancer\,unit\,risk$$

The objective of this calculation is to provide an estimate of the risk of developing cancer over a lifetime. The equation assumes essentially continuous exposure for a full lifetime (typically standardized to 70 years). However, the duration of measurement of the E_L is often substantially shorter than this time period (often 1–2 years at most). In some cases, modeling approaches can be used to derive a better estimate. In many situations, the result of the calculation will be relatively conservative, although this is often justified on the basis of the severity of the cancer effect. Adding to the conservatism of the calculation is the fact that cancer unit risks (aka slope factors) are typically developed as upper-bound estimates in order to take into account sensitive population groups. There are several additional important features of this analysis:

- These calculations are designed as statistical projections of hypothetical risk and are intended as screening tools. They cannot be used to make realistic predictions of actual biological effects in an individual or a population.
- These calculations cannot be used to determine if somebody who already has cancer developed the disease because of past exposures.

- Excess cancer risks are usually expressed as individual risks i.e. the risk born by an individual belonging to a larger population.
- The number of cases of cancer expected over a lifetime can be calculated by multiplying the cancer risk to an individual by the number of individuals. Critically, the results of this calculation may indicate a low predicted cancer incidence rate, however *this does not mean that individuals within the population will not develop cancer because of exposures to the chemical concerned*.

When mixtures and common cancer modes of action/cancer pathways are involved, an approach that is analogous to the RI is used and an additive interaction between different carcinogens in a mixture is assumed:

$$Risk_{Total} = Risk_1 + Risk_2 + Risk_3 + \cdots$$

This screening-level approach assumes a linear dose-response and act via similar pathways. Thus the effects of individual chemicals within a mixture are regarded as being additive. However, more refined methods take into account issues of synergy, antagonism, different pathways and so forth. When more than one pathway is involved, the pathway specific risks are summed and then all the risks are summed across the different pathways. Please note that non-classically mutagenic carcinogens (i.e. do not directly act on DNA and act via epigenetic, hormonal or redox pathways) are generally regarded as not having a linear dose-response. The RQ and RI approaches are usually used for these materials.

The metric "mortality" is defined as the number of excess deaths that will result over a specified time period due to the presence (or absence) of the risk or exposure of interest. Thus it contains two attributes: the exposed population and the number of deaths.

The mortality rate is the frequency of occurrence of death in a defined population during a specified interval:

$$Mortality\ rate = \frac{Deaths\ occuring\ in\ a\ given\ time\ period}{Size\ of\ the\ population\ at\ the\ midpoint\ of\ the\ time\ period} \times 10^n$$

where 10^n is often 1000 or 100,000. A variety of mortality rates can be calculated:

- Crude death rate (CDR) is the mortality rate from all causes of death for a population.
- Cause-specific death rate (CSD) is the death rate attributed to a specific cause or risk factor for a population i.e.

$$CSD = \frac{Number\ of\ deaths\ due\ to\ a\ specific\ cause\ over\ a\ specific\ time\ period}{Population\ size\ at\ the\ mid\ point\ of\ the\ time\ period} \times 100{,}000$$

- Age-specific mortality rates are mortality rates that are limited to a specific age group.
- Infant mortality rate (IMR):

$$IMR = \frac{Number\ of\ deaths\ among\ children < 1\ year\ of\ age\ over\ a\ specific\ time\ period}{Number\ of\ live\ births\ reported\ during\ the\ specific\ time\ period}$$

Similar calculations can be made for neonatal mortality rate, post-neonatal mortality rate and maternal mortality rate. It is important to note that the IMR is not a proportion because some of the deaths in the numerator were reported in the previous year e.g. some of the deaths recorded in a 2013 IMR will, in fact, have been born in 2012 where as the denominator only includes children born in 2013. Also, the IMR is not really a rate because the denominator is not the size of the relevant population at the midpoint of the relevant time period.

- Sex-specific mortality rate.
- Race-specific mortality rate.
- Various combinations of specific mortality rates.

The death-to-case ratio (DCR) is:

$$DCR = \frac{Number\ of\ deaths\ attributed\ to\ a\ specific\ cause\ over\ a\ specific\ time\ period}{Number\ of\ new\ cases\ of\ disease\ due\ to\ a\ specific\ cause\ over\ a\ specific\ time\ period}$$

The standardized mortality ratio is calculated by:

$$SMR = \frac{Observed\ Number\ of\ Deaths\ per\ Year\ with\ Exposure}{Expected\ Number\ of\ Deaths\ per\ Year\ Without\ Exposure} \times 100$$

where the expected death rate is derived from a matched, non-exposed, control population. Typically, a 95 % confidence interval and a *p* value for the SMR are also calculated. A SMR of above 100 means the number of observed deaths is greater than what would be expected if the study population had the same probability of dying as the standard population, while a SMR of below 100 means the number of observed deaths is less than expected. The SMR technique is a form of indirect standardization. One of its advantages is that allows for age-adjustment when age stratification may not be available for the cohort being studied i.e. the test and control cohort populations can be selected to have the same age range (i.e. age-specific SMRs), and other potentially confounding variables can be controlled for in a similar manner. It is also possible to standardize for known exposure periods (i.e. a time period SMR), known exposure level (exposure level SMR) and to combine different components of standardization (e.g. generate a age-specific and time period-specific SMR). SMRs have a couple of kinks to be aware of:

- SMR studies with worker populations classically display the "healthy worker effect," thus occupational studies generally have SMRs < 100. The reason for this is that workers tend to be healthier than the general population that contains both healthy and unhealthy individuals.
- *You cannot compare SMRS across different studies! You can only compare SMRs to the standard population.*

The SMR methodology has the advantage of controlling for homogeneous subgroups and providing detailed information. However the technique can become cumbersome if there are many subgroups or complex population sub-divisions.

An extension of the SMR concept is the proportionate mortality ratio (PMR):

$$PMR = \frac{Deaths\ due\ to\ exposure\ to\ the\ risk\ factor}{Deaths\ from\ all\ causes} \times 100$$

A PMR of > 100 indicates that a particular risk factor accounts for a greater proportion of deaths in the population of interest than might be expected. Like the SMR, factors such as age group, exposure period, exposure level and so-forth can be factored into the analysis. PMRs have the same kinks as SMRs, namely the healthy worker effect and lack of comparability across studies.

Morbidity is an attribute that refers to ill health, but not death, associated with exposure to a risk factor. In mathematical terms, morbidity can essentially be substituted anywhere you can use mortality. It is simply a choice regarding what effect(s) are of interest.

Disability-adjusted life years (DALYs) represent the loss of 1 year of "healthy" life. If DALYs are summed across an entire population or across a specific disease burden, the result is a measurement of the gap between the current health status and an idealized health outcome (i.e. the entire populations lives to an advanced age, free of disease and disability). DALYs are calculated as:

$$DALY = YLL + YLD$$

Where YLL is the years of life lost due to premature mortality and YLD is the years lost due to disability for people living with the disease and/or its consequences. YLL is calculated as:

$$YLL = N \times L$$

Where N is the number of deaths attributable to the disease and L is the standard life expectancy in years. Note that YLL measures an incident stream of lost years of life due to disease-related death. YLDs are calculated as:

$$YLD = I \times DW \times L$$

Where I is the number of incident cases, DW is the disability-weighting factor and L is the average duration of the case until remission or death (in years). The DW ranges from 0 (perfect health) to 1 (death) depending on the severity of the disability.

YLD can also be calculated as prevalence[1] (prevalence YLD) as:

[1] Incidence is a measure of the risk of developing a condition within a specified period of time. In the case of YLL and YLD, the time period is one lifetime. Prevalence, on the other hand, is the proportion of the total number of cases to the total population. Prevalence is a measure of the

$$YLD_{Prevalence} = P \times DW$$

Where P is the number of prevalent cases.

Quality adjusted life-years (QALYs) are measure of the burden of disease that take into account both the quality and quantity of life. Essentially, QALYs are a measure of the value of health outcomes. The basic mathematical underpinning of the QALY is:

$$1\,QALY = 1\,year\,of\,life \times 1\,utility\,value$$

where 1 QALY is 1 year of life lived in perfect health. The utility value (UV) is an attempt to quantify the quality of life e.g. a UV = 1 means perfect health, a UV of 0.5 means alive by bedridden. The UV value is an interval scale with 0 being an arbitrary value meaning death. UV values are often determined by 3 techniques:

- Time-trade-off (TTO): how much life span would a person in a state of ill health is willing to trade off in order to be restored to perfect health, but with a shorter lifespan.
- Standard gambling (SG): persons in a state of ill health are given the choice of a medical intervention that has a chance of restoring them to perfect health, or killing them.
- Visual analogue scale (VAS): members of the population in question are asked to rate a state of ill health on a scale from 0–10 with 0 representing being dead and 100 representing perfect health.

QALYs are also frequently used to measure the impact of risk management procedures. Within this context, some form of incremental cost effectiveness ratio (ICER) is calculated by:

$$ICERR = \frac{Cost\,of\ Risk\ Management\ Procedure}{\Delta\,QALY\ produced\ by\ the\ Risk\ Management\ Procedure}$$

DALYs and QALYs have been subject to significant criticism because both techniques require a set of embedded value judgments about different levels of health impairment. Often such judgments are imposed by the personal performing the risk assessment rather than being derived from the "bottom up" i.e. from the people who are actually suffering the health impairment. Furthermore, the QALY calculation, while seemingly simple, actually becomes mathematically quite complex because life-years are expressed in a ratio scale with a true absolute zero, where as UV is measured as an interval scale with an arbitrary value for zero. The different nature of the two components of the QALY impacts upon the meaning and interpretation of QALYs. The mathematical solution to this limitation is via an alternative method

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burden of the disease on society irrespective of the time at risk or the period/timing of exposure to a putative risk factor. Usually, incidence values are more valuable given that they allow for the detection of associations between changes in incidence and changes in some putative risk factor.

for calculation of QALYs using complex numbers rooted in Pythagorean theorem (which is beyond the scope of this chapter). DALYs and QALYs also present a challenge in terms of risk communication: "reducing different kinds of hazard to a common metric (such as fatalities per year) and presenting comparisons only on that metric have great potential to produce misunderstanding and conflict and to engender mistrust of expertise (NRC 1989)."

Personal controllability (PC) is an attribute that describes the degree to which an individual can avoid or reduce their risk by voluntary action. Personal controllability is most important in risk perception and risk-acceptability and feeds into the concepts of voluntariness and controllability. Personal controllability is affected by 3 main factors:

- Awareness of the risk and potential for harm.
- Availability of options for avoiding, eliminating or reducing risk.
- Knowledge of the risk reduction/avoidance options and the ability to choose one.

Ability to detect health effects (ADHE) is a measure of the ability of informed institutions (e.g. regulatory agencies) to detect (potential or real) population-level adverse effects. ADE is often affected by how well a risk or hazard is understood, and the capacity to detect and accurately measure the risk and/or hazard. Risks/hazards with long latencies or that are rare commonly have low ADE values. ADE also links into the concepts of sensitivity, specificity and predictive values (Fig. 5.1).

The final metric that is commonly considered it the ability to mitigate adverse health effects (AMAHE). This refers to the ability of institutions or users to manage, reduce or control any expected adverse health effects e.g. by the recommending use of personal protective equipment when spraying pesticides; or by product recall if some error or new information is detected. AMAHE is dependent on factors such as controllability (both personal and institutional), reversibility and how easy (and how expensive) it is to reduce risk. An important concept regarding this metric is the concept of the Swiss cheese model of accident causation (Reasons's accident causation model; Fig. 5.2; Reason 1990):

An ideal system is akin to a stack of Swiss cheese slices. The holes in each slice represent opportunities for a risk mitigation strategy to fail. Each of the slices is a defensive layer in a risk mitigation process. Hopefully, a failure may allow a problem through a hole in one or two layers, but because the holes in the different layers

		Condition		
		Condition Positive	**Condition Negative**	
Test Outcome	**Test Outcome Positive**	**True Positive**	**False Positive** (Type 1 Error)	$\text{Positive Predictive Value} = \frac{\sum \text{True Positive}}{\sum \text{Test Outcome Positive}}$
	Test Outcome Negative	**False Negative** (Type 2 Error)	**True Negative**	$\text{Negative Predictive Value} = \frac{\sum \text{True Negative}}{\sum \text{Test Outcome Negative}}$
		$\text{Sensitivity} = \frac{\sum \text{True Positive}}{\sum \text{Condition Positive}}$	$\text{Sensitivity} = \frac{\sum \text{True Negative}}{\sum \text{Condition Negative}}$	

Fig. 5.1 The relationships between sensitivity, specificity and predictive values

Fig. 5.2 The Swiss cheese model of accident causation. (Reason 1990)

are in different places, the overall risk mitigation strategy does not fail catastrophically. For the risk mitigation strategy to fail catastrophically, the holes in each successive defense layer must line up. Such a risk mitigation strategy is inherently flawed. The more layers of defense, the smaller the holes, and the less "lined up" the holes are, the less likely that catastrophic failure will occur.

A Stepwise Process For Performing a Risk Characterization

Planning and Scoping The objective of planning and scoping is to define the purpose of a risk characterization, to focus on the critical conclusions of the risk assessment and to develop a coherent picture for applying and communicating the results of the assessment. A useful typology for planning and scoping of the types of risk characterizations encountered has been developed (NRC 1996):

- "Unique, wide-impact decisions and risk characterizations. The risk characterization informs single-time decisions that uniquely impact the health of large numbers of people or large portions of the environment, sometimes over long periods of time. Typically, they are controversial, with disparate perspectives on the nature and extent of the risk and a spectrum of affected parties and visible, interested stakeholders. Those planning the risk assessment process will no doubt recognize and have the

support for extensive risk analyses with broad participation. But the nature of the process will be particularly important in achieving a risk characterization that will be useful in the decision-making process."

- "Routine, narrow-impact decisions and risk characterizations. Risk characterizations of this type will be very similar to previous ones that have been performed. Typically, the impact under review will involve a small geographical area and few people. Significant unresolved issues may underlie individual risk characterizations of this type. However, it will be neither practical nor desirable to debate the assumptions and develop multiple descriptions for each risk characterization. The most reasonable course is to make the process and characterization development routine, but provide the opportunity for appeal. Also, there should be periodic review of the routine procedures."
- "Repeated, wide-impact decisions and risk characterizations. Risk characterizations of this type have wide impact; that is, they support decisions that can have an impact on large numbers of people or large geographical areas. However, the characterizations developed are similar in structure to ones done previously with respect to issues discussed and supporting risk assessments. Also, in planning and scoping the assessment process, the issues are likely to be similar to those previously raised. Therefore, some aspects can be made routine, although certain other aspects may need special attention so that they meet the unique needs of the particular decision at hand. Also, questions should be raised at the start to attempt to uncover issues important to the decision that would not be anticipated on the basis of other similar risk characterization exercises."
- "Generic hazard and dose-response decisions and risk characterizations. Risk characterizations of this type are one step removed from the characterization of a particular chemical use or site-specific risk. In fact, they typically support the routine risk characterizations described above. Since they fall outside specific decisions at hand, it is sometimes difficult to appreciate the full range of issues. Indeed, it may be a challenge to construct a risk assessment or characterization development and review process with adequate participation, absent a particular decision context."

Elements of a risk characterization: The process of conducting a risk assessment will usually identify any necessary policy issues, uncertainties as well as the risk assessment conclusions. The overarching objective of the risk characterization phase *is not to repeat the entire risk assessment process*. The holistic objective is to describe the *key* findings from each step of the assessment and how these were discovered during the process. The elements of a typical risk characterization will include:

- Key information: the objective is to capture the key information from the risk assessment and carry this forward into the risk characterization. The important considerations are: (a) what studies are available and their robustness; (b) what is being assessed (including which population[s]), the major risk estimates calculated, any assumptions and/or extrapolations associated with the calculations, any residual uncertainties (and their impact on the risk estimates); (c) the use of default approaches or values, any policy choices and any risk management

decisions made; (d) The level of acceptance of the key data e.g. is the data experimental, state-of-the-art or generally accepted?; (e) presentation of the quantitative data in a readily understandable form i.e. the use of tables and graphics; and (f) variability.

- The context: The objective is to place the current risk assessment into context with similar risk assessments in order to develop a sense of the comfort level associated with the assessment, the weight of evidence, and the likely problems relating to acceptance. Often comparing and contrasting the current risk assessment with similar ones performed by other agencies and individuals can accomplish these objectives.
- Information on sensitive subpopulations: any sensitive populations evaluated by the risk assessment should also be appropriately characterized.
- Scientific assumptions: Information on the scientific assumptions is an inevitable component of risk assessments. It is critical that these assumptions (many of which are enshrined in various agency guidelines and policies) are clearly delineated.
- Likely policy choices: different agencies (or even divisions within the same agency) have different policies pertaining to risk assessment. There are also generally accepted policies and approaches for conducting risk assessments under specific sets of circumstances. It is important that any policy choices be consciously made (i.e. not just blind adherence to defaults), described and justified.
- Variability: it is important to distinguish between variability and uncertainty. Variability is an important biological trait that results from true heterogeneity within a population. Variability will change depending on the population examined and the circumstances of the risk assessment. It is usual to provide measures of central tendency and high-end individual risk descriptors that are important in the distribution of risk across a population.
- Uncertainty: uncertainly is "we don't know what we don't know" and it is imperative to separate this concept from variability. Uncertainty arises from a lack of knowledge (i.e. data gaps, lack of knowledge about mode/mechanism of action etc.) and measurement uncertainty (including modeling uncertainty). The only way to address it is to increase knowledge and perform better experiments/modeling. Quantitative assessments of uncertainty are generally preferable, however even subjective information is valuable. Currently there is no general consensus on how to conduct an uncertainty analysis but this is no excuse for not conducting one. Additionally, uncertainty analysis should, at a minimum, cover issues pertaining to precision, accuracy, data gaps, modeling and its limitations, the dose response assessment, the exposure assessment and uncertainties generated by any scientific assumptions/policy choices. It is particularly important to clearly identify any assumptions and/or policy that would make a big impact on the risk assessment.
- Bias and Perspective.
- Strengths and Weaknesses.
- Key Conclusions: A useful approach is to ensure that each section of a risk assessment has its own summary "mini-characterization." This provides a ready-formulated set of key points to be communicated in the risk characterization section. However, the objective here is not to produce a verbatim recitation of

each "mini-characterization." Rather, the aim is to convey a small subset of **key** findings, strengths and limitations that make a difference in the outcome. It is important to use plain English, to follow TCCR principles, provide a brief bottom line regarding the risks and the level of confidence in the risk assessment. A paramount requirement is to clearly convey what is known about the nature, likelihood and magnitude of the known risks.

- Alternatives Considered: The important messages are: (a) what are the alternatives to the hazards assessed and how do they compare?; (b) place the risk findings in context i.e. how to the risks found in this analysis compare with other known risks (including those of any similar agents, alternative replacement agents and common risks faced by the relevant population); and (c) what are the limitations of any comparisons made
- Research Needs: The aim here is not to list or describe every single possible research option. Rather, the objective is to identify key data/methodology gaps and address the areas of proposed new research that will really make a difference. Ideally, a relative priority for any suggestions made for future research and development should be made. An important issue may arise from this analysis: should the risk assessment and key decisions be delayed until additional research is completed? Certainly, if the research need is a compelling requirement for the risk assessment to move forward, a case can be made for a delay. A compelling requirement might be the need for a greater understanding of a mode of action, or a greater understanding of susceptible populations and so forth. Essentially, it is a clear recognition of a critical scientific data gap that seriously impacts the outcome of a risk assessment/risk characterization.

Using "bright lines" or "magic numbers" in risk characterization: this type of approach should be avoided. Bright lines and magic numbers are oversimplified scientifically reductionist numerical thresholds that indicate whether a risk is acceptable or not. Good risk characterization is not amenable to this type of scientific reductionism: "the goal is to give an understandable, rich description of the findings and the strengths and weaknesses of the assessment. "Every risk characterization has a fundamental, irreducible set of information consisting of the key findings that must be conveyed to every audience to adequately characterize the risk; again, it is more than just a number (Fowle and Dearfield 2000)."

Informing Decision Makers

This step requires an understanding of what is driving the decision making process. The decision driving factors will vary on a case-by-case basis. However, a typical set of factors will include:

- Scientific factors: this is essentially the content of the risk assessment.
- Economic factors: usually this is reduced to some form of cost: benefit analysis i.e. how much benefit would be gained by implementing the findings of the risk assessment versus how much cost will be involved.

- Laws and legal decisions.
- Social factors and public values: no matter what the quality of the risk assessment, it stands no chance what so ever of being implemented if the population concerned do not accept it. The old adage "you can lead a horse to water, but you cannot make it drink" is applicable here. Top-down decisions that are enforced against community beliefs usually meet with substantial resistance and poor compliance. Often a better approach is to educate communities and help them comply. Even if a risk assessment is rejected because of social factors, the risk assessors should take comfort in the fact that they have helped the affected communities to at least be accurately informed.
- Technological factors: these include the practical feasibility, impact and range of risk management options.
- Political factors: risk assessors should be aware that persons involved in the political sphere (this includes people *other* than politicians as well) commonly have very different priorities than risk assessors. Paramount amongst these priorities are: to at least be *seen to be informed,* to be *seen to be in charge,* and to be *seen to be adhering to some set of ideological principles.*

Managing the relationship with decision makers will frequently involve the capacity to argue successfully and to interpret the validity of any counter-arguments. A useful approach to understanding the ways in which risk characterizations are attacked and criticized by non-technical management structures and non-technical stakeholders (and how to respond and deal with such arguments) is to apply Graham's Hierarchy of Disagreement (Fig. 5.3).

Of all the different phases of performing a risk characterization, this step is often the one that with either make or break the outcome in terms of acceptance. Risk assessments and risk characterizations are commonly met with a barrage of criticism. It is critical for those who perform risk characterizations to understand the types of attack and counter arguments they face, and how to respond to them, in order for the risk assessment and risk characterization to be accepted and acted upon.

In general, stakeholder counter arguments and responses to the risk characterization/risk assessment that involve the lower levels of Graham's Hierarchy (i.e. name-calling, *ad hominem*, responding to tone, and contradiction) are *not an adequate basis for altering the conclusions of the risk characterization and risk assessment.* Stakeholder counter arguments and responses to the risk characterization/risk assessment that involve the upper levels of the hierarchy (i.e. counterargument, refutation, and refuting the central point) are critically important parts of the scientific process and must be dealt with in a thorough and scientifically credible manner. Such well-formed counter arguments may indeed be a necessary indicator that further thought and work on the risk characterization and risk assessment needs to be done!

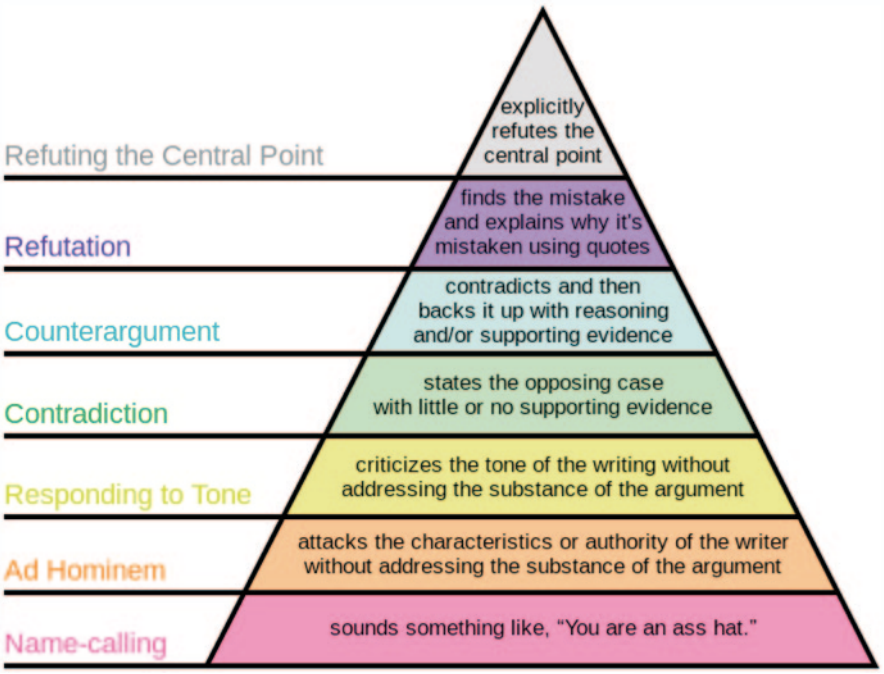

Fig. 5.3 Graham's hierarchy of disagreement

Chapter Summary

This chapter addresses the following knowledge areas that are relevant to the process of risk characterization: (a) the role of risk characterization in the risk assessment process; the overall holistic purposes of a good risk characterization; (c) the principles of transparency, clarity, consistency and reasonableness; (d) how a risk characterization is used; (e) the various metrics that may be used in a risk characterization; (f) the relationships between sensitivity, specificity and predictive values; (g) introduces Reasons's accident causation model; (h) describes a stepwise process involved in developing a risk characterization; (i) highlights common factors that drive decision-makers; and (j) introduces Graham's hierarchy of disagreement.

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Chapter 6
Risk Assessment Strategies and Techniques for Combined Exposures

Cynthia V. Rider and Jane Ellen Simmons

Abstract Consideration of cumulative risk is necessary to evaluate properly the safety of, and the risks associated with, combined exposures. These combined exposures ("mixtures") commonly occur from exposure to: environmental contaminants in air, soil, and water; pharmaceuticals and dietary supplements; consumer and personal care products; food additives and residues; and nonchemical stressors (e.g., physical and psychosocial). Risk assessments of mixtures of chemicals are more complex than those of single chemicals for two major reasons: (1) in combining chemicals to estimate mixture risk, it is necessary to rely on multiple assumptions; and (2) the potential for pharmacokinetic and/or pharmacodynamic interactions among mixture components. Additional difficulties exist for complex environmental mixtures, which typically contain a large fraction of total mixture mass of unknown identity and toxicity. The influence of data type, quality and quantity on the risk assessment approach is illustrated. Guidance is provided on when whole mixture risk assessment approaches are possible and when component-based approaches are needed. Advantages and disadvantages of whole mixture risk assessment approaches are discussed, including concerns due to unknown mixture mass and the current status of sufficient similarity methodology. Component-based methods based on dose-addition represent the majority of chemical mixture risk assessments that have been conducted to date; both hazard index-based (Hazard Index, Target Organ Toxicity Hazard Index, Interaction-Weighted Hazard Index) and index chemical (Relative Potency Factor and Toxic Equivalency Factor) approaches are reviewed. There is recognition of the need to consider the cumulative effects of both chemical and nonchemical stressors, but standard methods with a history of use are not available.

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Keywords Chemical mixture · Dose addition · Response addition · Whole mixture · Defined mixture · Cumulative risk assessment

Student Learning Objectives

- Understand the motivating factors for chemical mixture risk assessments and cumulative risk assessments
- Understand the definitions of additivity and be able to articulate the difference between dose addition and response addition
- Understand how risk levels may change under additive, greater than additive and less than additive interactions
- Be able to calculate a Hazard Index and a Target Organ Toxicity Hazard Index
- Be able to explain the application of toxic equivalence factors and relative potency factors
- Understand the difference between Cumulative Community-based Risk Assessments and Cumulative Disease-Based Risk Assessments

Introduction

Most risk assessments have been performed on single chemicals. However, people are routinely exposed to complex combinations of stressors, including multiple chemical and nonchemical stressors (i.e., biological, physical, and psychosocial stressors). Therefore, it is important to consider combined exposures in risk assessment. This goal is reflected in an ongoing expansion of the coverage of risk assessments from more traditional single-chemical risk assessments to cumulative risk assessments that include chemicals within a defined class (e.g., organophosphate pesticides) to community-based risk assessments that attempt to consider the totality of exposures experienced by a given community, including both chemical and nonchemical stressors. This chapter will address definitions, concepts, and methods used to assess risk from combined exposures, including exposure to multiple chemicals and combined exposure to chemical and nonchemical stressors.

Risk assessment for chemical mixtures differs from single chemical risk assessments because the risk assessor must be aware of the potential for interactions among the chemicals comprising the mixture. A key issue is that mixtures risk estimates can change depending upon which assumptions are used to define the baseline additive scenario against which interactions (between chemicals and/or between

chemical and nonchemical stressors) are identified. Dose addition and response addition are the established concepts for estimating the combined effects of chemicals. Predictions of risk based on an assumption of dose addition can differ dramatically from those based on an assumption of response addition. In turn, conclusions about directionality and magnitude of interactions can differ depending on the use of dose addition or response addition to estimate the baseline scenario.

Mixtures risk assessments focus on those mixtures of chemicals that enter the body as discrete chemicals. However, it should be recognized that once inside the body, a chemical that enters the body as an individual chemical can form mixtures. Xenobiotic metabolism, while principally carried out by the liver occurs to a lesser degree in kidney, lung, small intestine, heart, muscle and brain. Metabolism may result in the formation of multiple metabolites. Interactions are possible between the metabolites and the parent compound. One of the best studied examples is that of n-hexane which is metabolized within the liver to form methyl n-butyl ketone which in turn is metabolized to form 2,5-hexanedione (the proximate neurotoxicant). n-Hexane inhibits the oxidation of methyl n-butyl ketone to 2,5-hexanedione, explaining the apparent paradox that higher concentrations of n-hexane result in lesser neurotoxicity than lower concentrations (Andersen and Dennison 2004). However, to date there are no known cases of a mixtures risk assessment accounting for a chemical that enters the body as a single chemical but is metabolized to chemicals that either interact with the parent compound or each other.

Overwhelmingly, cumulative risk assessments are conducted for defined mixtures. Defined mixtures are those where all the component chemicals are known. This may be contrasted with highly complex mixtures detected in the environment where incomplete chemical characterization of the mixture is the rule, i.e., significant portions of the mixture mass are not known. Defined mixture exposures can be concurrent or temporally separated and can occur via the same or differing routes of exposure. Examples of defined mixtures with concurrent exposure by the same route include antimicrobial mixtures and cancer chemotherapy cocktails. Examples of defined mixtures where exposures are typically temporally separated include exposure to ethanol during off-work hours and occupational exposure to industrial solvents. Defined mixtures where exposure is both concurrent and temporally separated include the regulated trihalomethanes and haloacetic acids formed during oxidant disinfection of water containing natural organic matter. These latter exposures are especially interesting as they are also multi-route exposures (e.g., exposure to the trihalomethane bromodichloromethane occurs by oral, inhalation and dermal routes). Additionally, they illustrate an important point, that the defined mixtures for which the risk assessment is being conducted may ‘reside’ within much more complex mixtures. With regard to the regulated trihalomethanes and the regulated haloacetic acids, they represent only part of the halomethanes and haloacetic acids that have been identified in drinking water. In fact, more than 600 unique compounds have been identified as drinking water disinfection byproducts but despite this intense identification effort, substantial portions of the total organic halide formed during water disinfection remains unknown (Fig. 6.1), (Richardson et al. 2008).

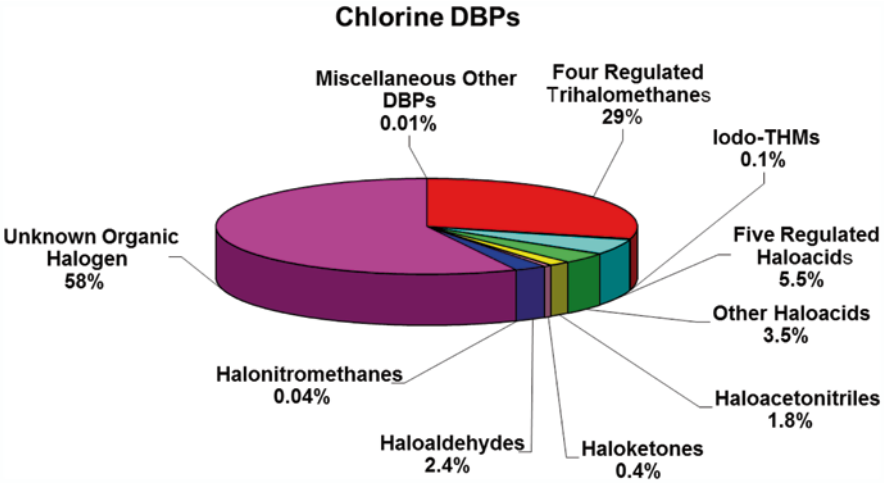


Fig. 6.1 Separation of the total organic halide formed during disinfection of drinking water by chlorine into known and unknown disinfection byproducts. Integrated disinfection by-products mixtures research: Comprehensive characterization of water concentrates prepared from chlorinated and ozonated/postchlorinated drinking water. (Adapted from Richardson et al. 2008)

Definitions

Due to considerable confusion with definitions key to understanding the potential human health consequences of exposure to mixtures of chemicals, it is important that these terms be clearly defined and used consistently within the risk assessment process. It is especially important for the risk assessment to be grounded in clear definitions of additivity and non-additivity, and that these definitions are consistently applied throughout the process. This confusion has hindered advances in toxicology, epidemiology and risk assessment of chemical mixtures and cumulative exposures. To avoid confusion within this book chapter, definitions for key words and concepts are provided here. This does not discount the usefulness of alternative definitions, but does highlight the need for authors to define, within their own work, words for which varied definitions are in use or have been used. The definitions that will be used in this chapter are derived principally from EPA guidance (US EPA 1986, 2000). The terms used to describe the interactive effects of chemicals, such as synergy and antagonism, have different meanings to different investigators and disciplines (see for example, Hertzberg and MacDonell 2002; Simmons 1995). This has created considerable confusion within the field. However, these terms can be avoided and clarity achieved if a clear definition of additivity (either dose or response) is provided and results and conclusions are considered as to whether they are consistent with additivity (no detectable deviation from additivity), greater than additive or less than additive. When terms such as synergy cannot be avoided, they should be defined in relation to the specified model of additivity being used.

Dose Addition Under dose addition, the effect (response) of the mixture is predicted by *summing the exposure doses or concentrations of the component chemicals*. A key concept is that the concentrations of the component chemicals are weighted by their toxic potency. In the idealized situation, the component chemicals behave as concentrations or dilutions of each other. Dose addition is thought to be best applied to those chemicals that *share a common or similar mode of action or similarity of target organ*. Thus, the behavior of a chemical mixture is considered dose additive if the effects of the combined components (i.e., the effect of the mixture) can be estimated from the sum of the scaled concentrations of the individual components. When the effect of the mixture is greater than expected, the risk associated with exposure to the mixture is increased. Conversely, when the effect of the mixture is less than expected, the risk associated with exposure to the mixture is decreased.

Response Addition Under response addition (also called independent joint action or independent action), the effect (response) of the mixture is predicted by *summing the effects (responses) of the component chemicals*. A key concept is that the mixture response is predictable by the sum of the responses of the components using the formula for the sum of the probabilities of independent events. Response addition is thought to be best applied to mixtures of chemicals that have *dissimilar modes of action*; these chemicals are toxicologically independent (i.e. the biological response to each chemical is the same whether or not the other chemical(s) are present. Thus, the behavior of a chemical mixture is considered response additive if the effects of the combined components (the effect of the mixture) can be estimated from the sum of the scaled responses of the individual chemicals. When the effect of the mixture is greater than expected, the risk associated with exposure to the mixture is increased and, conversely, when the effect of the mixture is less than expected, the risk associated with exposure to the mixture is decreased. The concept of response addition differs from **effect summation**, which represents a simple summation of component effects. Although effect summation is commonly used in the mixtures literature, it is generally not considered to be an appropriate method for defining additivity.

Synergy and Antagonism It is highly recommended that use of the terms "synergy" and "antagonism" be avoided due to the vast confusion that has plagued chemical mixtures toxicology and risk assessment because of the many differing definitions of these terms and their widespread use without articulation of the meaning ascribed by the user. Rather, it is recommended that conclusions be drawn as to whether the response of the mixture in question is consistent with a *specific definition of additivity* as either (a) "no detectable deviation from additivity", (b) "greater than additive", or (c) "less than additive". The definition of additivity should be specific as to dose or response addition with appropriate reference to the underlying literature. To avoid confusion "synergy" is replaced with "greater than additive" and "antagonism" is replaced with "less than additive." When the term "synergy" cannot be avoided, it should be defined within the context of the definition of additivity being used.

Aggregate Exposure, Aggregate Risk The term aggregate is used here to indicate the summing of exposure for an individual chemical across all relevant routes,

so that the total dose to the target can be used to estimate the aggregate risk. For example, in the case of bromodichloromethane, multiple routes of exposure (oral, inhalation and dermal) make significant contributions to internal dose and contribute to the aggregate exposure and aggregate risk.

Cumulative Exposure, Cumulative Risk The term cumulative is used here to indicate consideration of more than one stressor (chemical or nonchemical) in an exposure or risk assessment. *Cumulative is notably distinct from aggregate and should not be used interchangeably. However, an exposure characterization or risk assessment can be both aggregate and cumulative*. It is an umbrella term that does not dictate the specific model used to assess cumulative risk and concepts of either dose addition or response addition can be used as a basis for the calculation of cumulative risk. It is important to note the distinction between the concepts used to describe joint action (dose addition and response addition) and the methods available for calculation of risk (e.g., hazard index, relative potency factors) that are built upon those concepts.

Selection of Risk Assessment Method

The first step in a mixture risk assessment is evaluation of the existing data. It is important to note that if data availability and quality are judged to be inadequate, it is not appropriate to continue on with either a qualitative or quantitative risk assessment. A key element of conducting a chemical mixtures risk assessment is selection of the methodology that will be used. The mixtures flow chart (Fig. 6.2),

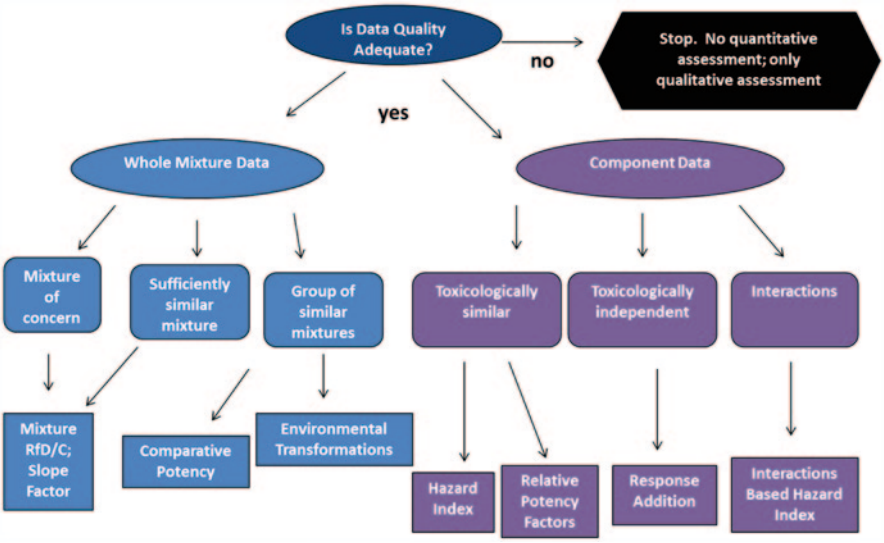


Fig. 6.2 Mixtures risk assessment flow chart. (Adapted from US EPA 2000)

illustrates whether it is appropriate to conduct a mixtures risk assessment and what type of methodology is appropriate, which is determined by the quality and type of data available. Thus, data availability and suitability determine the risk assessment approach. The preferred data are on the mixture itself. However, whole mixtures data are almost never available. When data on the mixture itself are not available, data on a 'sufficiently similar' mixture or group of similar mixtures are preferred. It is important to note that robust techniques to determine if two whole mixtures are sufficiently similar have not yet been developed, severely limiting the application of a sufficiently similar mixture approach. When neither data on the mixture itself nor data on similar mixtures are available, risk is assessed by component-based approaches in which single-chemical data, and information on simple, defined mixtures if available, are used. The majority of mixtures risk assessments employ component-based approaches.

As detailed in US EPA (2000) and outlined here, the quality of available exposure, health effects and interactions data are assessed with regard to completeness, relevance, nature (qualitative or quantitative) and certainty (Table 6.1). Each category of data (exposure, health effects, and interactions) is classified as good, fair or poor. Table 6.1 summarizes how consideration of the data quality is incorporated

Table 6.1 Data quality classification scheme[a]

Data Type		
Exposure		
	Good	Human exposure accurately or reasonably characterized (monitoring or modeling)
	Fair	Exposure estimates for some components are lacking/uncertain/variable but not likely to affect risk assessment; not all components identified or levels highly variable or uncertain and effect on risk assessment unknown
	Poor	Insufficient for quantitative risk assessment
Health Effects		
	Good	Full health effects data available and extrapolation if needed is minor or supported (e.g., pharmacokinetic, empirical observation)
	Fair	Full health effects data available but extrapolation not directly supported by available information
	Poor	Lack of health effects information; quantitative risk assessment not possible
Interactions		
	Good	Assessment based either on the mixture of concern or a sufficiently similar mixture
	Fair	Quantitative interactions of all components are well characterized or assumption of additivity is justified
	Poor	Interaction information lacking or assumption of additivity not justified; quantitative risk assessment not possible

[a] Excerpted from U.S. EPA (2000)

into the Mixtures Flow Chart (Fig. 6.2) to guide the risk assessor to the appropriate risk assessment strategy.

In terms of exposure characterization, the best-case scenario (representing the ideal situation within the 'good' category in Table 6.1) would provide all the data necessary to fully characterize human exposure to the whole mixture of concern from the point where the mixture enters the environment to the point of human contact/exposure. Information would be available on: (1) the concentration of the mixture in the environment at the environment/human interface; the duration of exposure and the frequency of exposure; (2) the environmental transformation of the composition of the mixture over both space and time; (3) the degree to which the composition of the mixture is known, including the percentage of the mixture mass that has been chemically identified and the accuracy and reliability of the measurement techniques; and, (4) uptake, including the magnitude of human exposure either from direct measurement or modeled based on human exposure patterns and the bioavailability of the mixture from the environmental medium for the route of exposure of concern. As might be expected, such high quality mixtures data are quite rare (as they are for most single chemicals), so various types of extrapolation are typically necessary. Frequently occurring extrapolations are across species (e.g., from experimental animals to humans), exposure route (e.g. oral to inhalation, inhalation to oral, etc.) and exposure durations (e.g., acute to chronic or subacute to chronic).

On the health effects side, the most data-rich scenario (again, representing the ideal situation within the 'good' category in Table 6.1) would include human clinical or epidemiologic data directly on the complex mixture of concern in which the health effects of concern are linked directly to mixture exposure with dose-response data available for the exposure route of concern. Again, such high quality mixtures data are quite rare and extrapolations are often necessary. As with exposure data, frequently occurring extrapolations for health effects are across species (e.g., from experimental animals to humans), exposure route (e.g. oral to inhalation, inhalation to oral, etc.) and exposure durations (e.g., acute to chronic or subacute to chronic). Additionally, extrapolation from endpoints where data are available (e.g., binding to the estrogen receptor) to apical health effects of concern (e.g., in vivo estrogenic effects) could be necessary.

Interaction information is related to whether the observed or anticipated response of the mixture is greater than expected under a clear definition of either dose addition or response addition. It is important to note that the observed effects of a whole mixture, whether it is observed in humans or an experimental animal model, represent an integrated response of the organism to all constituents and components of the mixture, including any potential interactions (i.e., greater than additive or less than additive). Therefore, whole mixture data represent the highest quality classification of 'good' (Table 6.1). Data that includes sufficient evidence of a lack of interaction or quantification of all identified interactions among components is classified as 'fair'; while data indicating that interactions are likely without quantifying those interactions is classified as 'poor'.

A rating of 'good' for all three types of information would be the result of solid data being available for both exposure and toxicity (health effects and interactions)

which would allow the risk assessor to conduct a quantitative assessment directly on the mixture of concern (Fig. 6.2). In contrast, a rating of 'poor' for any of the three factors would probably result in a judgement that data quality is inadequate for a qualitative assessment, with the likelihood of a judgement of 'inadequate data' increasing as the number of factors rated 'poor' increases. Ratings of 'fair' drive the assessment away from whole mixture and toward component-based approaches.

Whole Mixture Techniques

Mixture of Concern

The most important point to remember with respect to risk assessment methods for the mixture of concern is that they 'mirror' single chemical risk assessment techniques because they treat the whole mixture as if it were a single chemical. The data quality and quantity requirements are greater for 'mixture of concern' risk assessment methods than for other mixtures risk assessment methods (e.g., component-based approaches). Both exposure and toxicity data, either human or experimental animal, must be available on the mixture of concern. Dose-response analysis is conducted directly on the mixture data, using single-chemical techniques, such as development of a reference dose/reference concentration (RfD, RfC) or a cancer slope factor. The reader is directed to the dose-response chapter for single chemical methods such as RfD, RfC, and cancer slope. Advantages are: (1) single-chemical risk assessment procedures are well-developed and have a larger user base than procedures specific to mixtures; and, (2) whole-mixture risk assessments have fewer uncertainties and assumptions than component-based mixture risk assessments. While the calculations are the same as for single chemicals, some additional assumptions are made, including: the composition of the test mixture mirrors or mimics the environmental mixture and that sensitive endpoints have been taken into account.

Disadvantages of whole mixture assessments are that the ability to extrapolate is limited without an understanding of those chemicals and/or interactions responsible for the observed toxicity and the influence of the unidentified fraction. Methods are being employed to estimate the relative contributions of the component chemicals to the observed toxicity of the mixture. While it has not yet been applied to a complex mixture with an unknown fraction, the Expected Component Contribution score, described by Hertzberg et al. (2013) is expected to prove useful for whole mixtures as well as defined mixtures.

The Expected Component Contribution is the percentage of the mixture toxicity expected from each component when the components are known or assumed to behave in a manner consistent with dose additivity. It is calculated, for each component, as the product of its relative potency factor (RPF, see below) and its mixture fraction, with the resulting scores scaled so the sum equals 100. For the seven carbamate mixture investigated by Moser et al. (2012) and Hertzberg et al. (2013), the proportions of the chemicals in the mixture (also called the mixing ratio) were

based on the sales of each carbamate in California in 2005. While carbaryl comprised 39 % of the mixture, its Expected Component Contribution score was 4.25 % whereas oxamyl comprised 13 % of the mixture but had an Expected Component Contribution Score of 38.4 %.

Typically, a large percentage of the total mass of highly-complex environmental mixtures is not known and this unknown mass comprises the unidentified fraction. For example, Simmons et al. (1988) examined the *in vivo* toxicity of 16 hazardous waste samples. These samples had undergone quantitative analysis for 50 chemicals. Despite this extensive chemical characterization, identified mass ranged from a low of 6 % to a high of 61 % (mean characterized mass = ~40 %) for the 10 wastes where water was less than 90 % of the sample mass. Thus, the lack of methods for estimation of the contribution to toxicity of the unidentified fraction has been rate-limiting for risk assessment of environmentally-realistic complex mixtures. A risk assessment-based approach developed by Rice et al. (2008) for assessing the toxicity of a complex mixture and the impact of the unidentified fraction is presented as a case study.

Case Study: Impact of the Unidentified Fraction in Whole Mixtures Rice et al. (2008) were interested in understanding the toxicity of complex mixtures of disinfection byproducts (DBPs), where a large percentage of the total organic halide is unknown (see Fig. 6.1). This example was chosen for a case study because, depending on which side of the flow chart is followed, either whole mixtures or component based risk assessment approaches can be used (Fig. 6.2). The authors used developmental toxicity data from two drinking water concentrates containing DBP mixtures to illustrate the strategy. The strategy relies on conventional component-based mixtures risk assessment approaches such as dose addition, response addition, and analyses of interactions. It is immediately clear that a complex process is involved in determining if some or all of the toxicity of a complex mixture is associated with the unidentified fraction. In working through Fig. 6.3, first, it must be determined that the mixture is toxic (yes to [1]) and the toxicity is not due to one component (no to [2]) and that toxicity is not due either to additivity (no to [3]) or nonadditive interactions among the known components (no to [4]). The authors emphasized the importance of consideration of contaminants, i.e., chemicals not associated with the actual environmental mixture. Step 6 focuses on the toxicity of the unidentified chemicals. This is in recognition that it is much easier to determine the portion of the observed toxicity that is due to one chemical (step 2), additivity (step 3), nonadditivity (step 4), and contaminants (step 5) and that it is only after accounting for these other sources of toxicity, that some portion of toxicity can be attributed to the unidentified fraction of the mixture.

Sufficiently Similar Mixture

Environmental mixtures are complex and dynamic and, as mentioned above, data are rarely available for mixtures of interest. Using DBPs as an illustrative example,

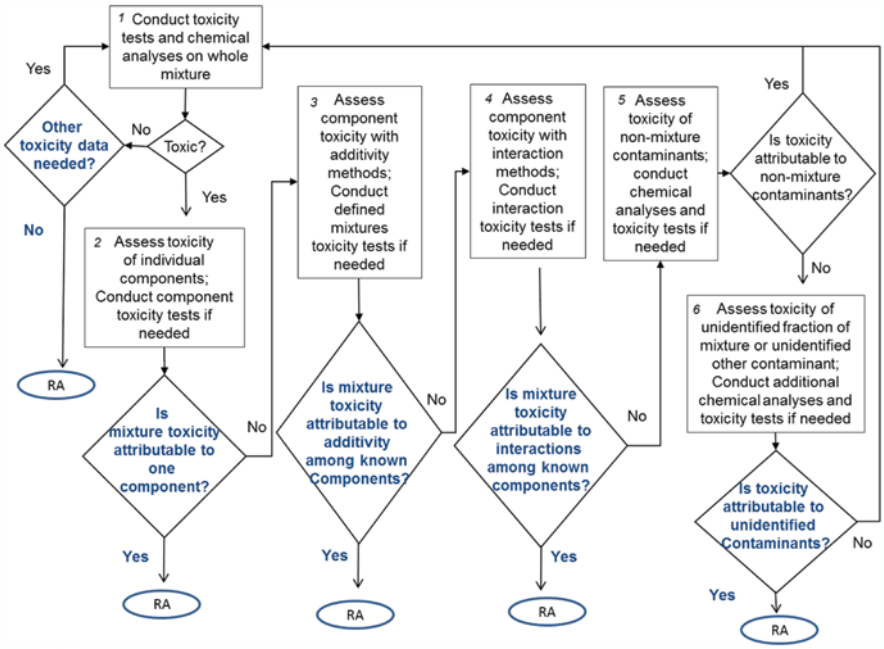


Fig. 6.3 Risk assessment (RA) based approach for assessing toxicity of a complex mixture and the impact of the unidentified fraction. Integrated disinfection by-products research: Assessing reproductive and developmental risks posed by complex disinfection by-product mixtures. (Adapted from Rice et al. 2008)

the components in the mixture and the ratio of those components will differ significantly based on factors such as source water (e.g., urban, rural), treatment method (e.g., chlorination, chloramination), the processing facility (e.g., engineering differences), environmental conditions (e.g., photochemical reactions, rain) and distance from the facility (e.g., dilution, additional contaminants). Therefore, sufficient similarity methods have been proposed to determine whether or not a mixture of interest is "sufficiently similar" to a reference mixture for which dose-response data are available (Rice et al. 2009). There are many considerations involved in selecting an appropriate reference mixture. For example, the reference mixture should be generated by the same process or collected from the same source as the mixture of interest. The reference mixture must be well-characterized—both in terms of chemistry and toxicity (i.e., robust dose-response data).

Once a determination is made that the reference mixture is sufficiently similar to the mixture of interest, data from this 'reference' mixture can be used to estimate the risk associated with the mixture of interest. Sufficient similarity approaches are in the development phase and have not been widely and consistently applied in risk assessment. This represents an active area of research. The following section is a discussion of methods available for assessing sufficient similarity of mixtures using DBPs as a case study.

Case study: Sufficient Similarity of Water Disinfection Byproduct (DBP) Whole Mixtures Treatment of drinking water with chlorine represents one of the most important public health breakthroughs in the twentieth century. However, one unintended consequence of the process is the formation of complex mixtures of DBPs (around 600 compounds identified to date), which result from reactions between oxidizing disinfectants such as chlorine and organic materials found in water (Schenck et al. 2009). It is not possible to comprehensively characterize and evaluate each DBP mixture. Therefore, the overall goal of the EPA project described in this case study was to sort DBP mixtures into sufficiently similar groups based on some subset of parameters to simplify the evaluation process. Sufficient similarity of DBP mixtures was assessed using both the chemical composition and the biological activity of the studied samples.

Defining Important Chemical Properties of Complex Mixtures There are many different measures that can be used as inputs from which to evaluate the chemical similarity of different complex mixtures. It is important to note that in characterizing the chemical composition of complex mixtures, there will always be some unidentified fraction (see example in Fig. 6.1). Therefore methods to determine chemical similarity of complex mixtures rely upon a defined subset of constituents within the mixture. A notable caveat to using chemical similarity from which to make a judgment of sufficient similarity of complex mixtures is that it is possible that one or more particular components associated with the biological activity or health outcome of interest are unknown and remain in the uncharacterized fraction of the complex mixture (see case study above on impact of the unidentified fraction).

1. *Factors known to affect DBP mixture composition*—Parameters included here can be incorporated into models to predict mixture composition. These factors include: source water attributes (e.g., total organic carbon, temperature, bromide, pH), water treatment methods (e.g., disinfectant employed, other treatments) and engineering of the distribution system (Bull et al. 2009b).
2. *Subset of components*—In the DBP case study, researchers asked whether a subset of chemicals (commonly-monitored trihalomethanes and haloacetic acids) could be used as correlates for chemical similarity of other DBP components by examining detailed chemical analyses from 35 water treatment plants (Bull et al. 2009a). They concluded that no single class of compounds served as an adequate representative for the complex mixtures of DBPs. Instead, composite measures that correlate well with biological activity, such as total organic halides, might be more helpful to determine sufficient similarity of mixtures.

Biological Similarity of Complex Mixtures When comparing across complex mixtures, whole animal assays are preferred because they include absorption, distribution, metabolism, and elimination. However, these data are rarely available and the overwhelming majority of work to compare biological similarity of complex mixtures has been conducted using *in vitro* assays (e.g., mutagenicity in bacteria, cytotoxicity in cells). An important consideration is whether the *in vitro* assays employed are representative of the appropriate *in vivo* health effects. A simple

approach for determining biological similarity is to assess various complex mixtures in parallel and evaluate the potency of each sample. In the DBP example, researchers evaluated the relationship between different water quality parameters and the level of mutagenicity in different samples from 5 water treatment facilities (Schenck et al. 2009). They found that the best correlation was between total organic halide (TOX) concentration and mutagenicity. This kind of study will help with future determinations of which parameters (e.g., mutagenicity and TOX) are needed to determine sufficient similarity among DBP samples. In addition to the input of these measures, the process of determining sufficient similarity and when reference mixture data can be used as a substitute, involves statistical modeling and some level of judgment by the risk analyst (Rice et al. 2009).

Statistical Methods for Determining Sufficient Similarity The EPA guidance on conducting health risk assessment of chemical mixtures lists several requirements for considering whether or not a mixture is sufficiently similar to a reference mixture or a group of reference mixtures (US EPA 2000). The components in the mixture, proportion of the components, and uncertainties associated with use of a surrogate reference mixture should all be considered in the process. However, specific statistical methods are not prescribed by the guidance document. Using the DBP case study, Feder et al. propose two statistical approaches that can be used to determine sufficient similarity and meet the criteria specified by the EPA: a multivariate statistical procedure (Feder et al. 2009b) and a bootstrap hypothesis test procedure (Feder et al. 2009a). The details of these procedures are beyond the scope of this chapter.

Component-Based Approaches

Component-Based Approaches Based on Dose Addition

Component-based risk assessment methods are the most commonly used to assess mixture risk and are also among the easiest of the available methods to employ. The basic formula for dose addition as given by the U.S. EPA (2000) is:

$$R_m = f_1\left(D_1 + t_2 * D_2 + \ldots + t_n * D_n\right)$$

where

R_m = mixture response
D_1 = exposure dose of chemical 1 and D_n is the exposure dose of chemical n
t_2 = potency of chemical 2 relative to chemical 1 and t_n is the potency of chemical n relative to chemical 1
f_1 = dose-response curve for the index chemical, chemical 1

The primary criterion for selection of dose addition as the additivity model is toxicological similarity (US EPA 2000). The dose addition methods currently used

often carry one or more of these assumptions: similarity of mode of action or mechanism of action; similarity of shape of dose response curves; that components have similar uptake and pharmacokinetic behavior; for equal effects, the dose of one component is a constant multiple of the dose of a second component; and, toxicological similarity (by default, same target organ can be interpreted as toxicological similarity). Primary advantages of component-based approaches are their ease and simplicity of use. An additional major advantage is that they require data only from the component chemicals contained in the mixture and not from the mixture itself. As single chemical data are much more abundant than mixture data, the need for only single chemical data enables significantly more risk assessments than would occur if data on the mixture itself or a sufficiently similar mixture were required. The key assumption in the use of the Hazard Index and the Target Organ Toxicity Hazard Index is that the combined effect/behavior of the individual components of the mixture is predictable under an assumption of dose addition.

Hazard Index An assumption underlying the Hazard Index is that the component chemicals have a common or similar mode of action. This assumption can be met by use of a surrogate measure of similarity—that of the same target organ. This relaxation of the similar mode of action assumption allows more chemicals to be considered in the Hazard Index approach, as delineation of the mode of action of the majority of chemicals remains either unknown or uncertain. The Hazard Index requires toxicity dose-response data and exposure data on the individual components. The exposure level of each component is scaled to a measure of relative potency; for example, by the Reference Dose (RfD) or 'acceptable level'. These scaled concentrations are then added to obtain an estimate of the mixture risk.

The equation for the Hazard Index is:

$$HI = \left(E_1/AL_1 + E_2/AL_2 + \ldots . E_n/AL_n\right)$$

Where

HI = Hazard Index

E_1, E_2, E_n are the exposure levels for chemical 1, 2 and n, respectively.

AL_1, AL_2, AL_n are the 'acceptable levels' for chemicals 1, 2 and n, respectively.

Note that both E and AL must be expressed in the same unit of measure (for example, mg/kg) so that the units cancel out; thus, HI is a unitless number. Additionally, all scaling factors (ALs) used to calculate a HI should represent the same measure of toxicity (e.g., they should all be the cancer slope factor or they should all be the LD_{50} or they should all be the ED_{10}). While great flexibility is allowed in selection of the measure of toxicity that is used for the scaling factor when calculating the HI, the requirement that they be the same measure of toxicity is strict, as is the need for the risk assessor to make clear the measure of toxicity used. For EPA, the most common scaling factor for oral exposures is the RfD and for inhalation exposures is the RfC (US EPA 2000). The smaller the HI is below 1, the less the concern with regard to increased risk from exposure to the mixture. As the HI increases relative to 1, concern with regard to greater risk increases.

E/AL is the equation for the Hazard Quotient (HQ) so the HI index equation can also be expressed as:

$$HI = HQ_1 + HQ_2 + \ldots. + HQ_n$$

Target Organ Toxicity Hazard Index The Target Organ Toxicity Hazard Index was developed based on recognition of the fact that the most common scaling factor for the HI is the RfD and that RfDs are based on the most sensitive target organ for a particular chemical. As an example, let us assume that we are considering the risk of a mixture of chemicals 1, 2 and n on the liver, as they all cause hepatic necrosis. While chemicals 1, 2 and n may all be liver toxicants, the RfD will not be based on liver toxicity unless the liver is the most sensitive target organ for each of these chemicals. For example, exposure to chemical 2 results in reproductive toxicity at 1 mg/kg/day and liver toxicity is not observed until 4 mg/kg/day. The RfD for chemical 2 is thus based on reproductive toxicity and not liver toxicity and calculating the HI to estimate the risk of hepatic effects from exposure to a mixture of chemical 1, 2 and n using the RfD as the scaling factor will overestimate risk. The Target Organ Toxicity Hazard Index avoids this risk overestimation by use of scaling factors that are specific to the endpoint or target organ for which the HI is being calculated. These toxicity specific scaling factors are called target organ toxicity doses (TTD). The equation for the Target Organ Toxicity Hazard Index is the same as for the HI, except that the TTD is used as the 'acceptable level' or AL. An advantage of not overestimating risk is that it allows remediation efforts to focus on those mixtures and exposures that present the greatest risk. The disadvantage is that TTD values for specific organs are not commonly available and those that have been derived are likely to not have undergone the rigorous development process inherent in the RfD.

Interaction-Weighted Hazard Index As the name indicates, the Interaction-Weighted Hazard Index (HIint) is intended to allow the risk analyst the ability to incorporate information on nonadditive interactions between/among the individual components of the mixture into the HI calculation and the consideration of the estimated health risk of the mixture. Thus, this method is not based on the key assumption of dose additivity that underlies both the HI and the Target Organ Toxicity HI. This is accomplished by modifying the HQ by using information on interactions among the components. These modified HQs are then summed to calculate the HIint. Lack of appropriate interaction data are rate-limiting for this approach. To date, this approach has had limited application as it has not been demonstrated with experimental data on binary interactions within a higher order (i.e. greater than two-component) mixture. Additionally, the approach will be limited to those situations where binary interaction data are available. Key assumptions are that: (1) binary interactions are more important for prediction of the toxicity of the mixture than higher order interactions; (2) interaction magnitude is not dose dependent; and (3) interaction magnitude is dependent on the mixing ratio of the mixture, i.e. the proportions of the component chemicals relative to one another. The assumption that

interaction magnitude does not depend on dose will typically restrict this method to the low dose/low response region, as there is a growing consensus that nonadditive interactions are dose-dependent. Other assumptions of this method are that: the interaction magnitude is greatest when the binary components are present in the mixture at equi-toxic dose levels; the HIint 'reduces' to the HI as the interaction magnitude decreases; and, the toxicologic effects of concern are limited to those induced by the component chemicals individually.

Relative Potency Factor The Relative Potency Factor method has proved to have wide utility and broad application since it was endorsed by the EPA in 2000 in the Supplementary Guidance for Conducting Health Risk Assessment for Chemical Mixtures (US EPA 2000). While both toxicity and exposure data are required, this method can be used where exposure data or surrogates of exposure are available for all components, but toxicity data are incomplete or missing for some component chemicals of interest. An index chemical is selected, which is usually the component chemical either with the most complete toxicity data or for which confidence in the quality of the toxicity data is greatest. The exposure concentrations of the other component chemicals are scaled relative to the potency of the index chemical. Relative potency factors are developed for specific exposure routes and specific toxic effects (e.g., the oral route of exposure and liver toxicity or the dermal route of exposure and kidney toxicity). Relative potency factors based on a specific toxic effect or exposure route should not be used for extrapolation to other toxic effects or exposure routes; rather, a new set of relative potency factors should be developed and implemented. The relative potency factor equation is:

$$C_m = \left(C_1 * RPF_1 + C_2 * RFP_2 + C_3 * RPF_3 + C_4 * RPF_4 + \ldots.. C_n * RPF_n\right)$$

Where

C_m = the mixture concentration expressed as the index chemical
C_1 = concentration of chemical 1 in the mixture, where C_1 = index chemical
C_2, C_3, C_4, C_n = the concentrations of chemicals 2, 3, 4 and n in the mixture
RPF = relative potency factor, which is 1 for the index chemical and which for any other component chemical can be greater than 1, equal to 1 (where the chemical of interest is judged equipotent to the index chemical) or less than 1.

The RPF method requires selection of the index chemical. For transparency in communication, a clear statement of the rationale for its selection should be included in the risk assessment. RPFs must be assigned to each component chemical, again providing a clear statement of the rationale for the selection of the RPF value for each component chemical. It is also important to define both the health endpoints and exposure routes covered by the RPF, with all other options excluded.

Toxic Equivalency Factors The Toxic Equivalency Factor (TEF) methodology is a special case of the RPF method. The principal difference between TEFs and RPFs

is that TEFs are considered applicable to all health endpoints associated with the chemicals under consideration and all exposure routes. The data needs and requirements are substantially greater for development of a TEF than for an RPF. The dioxins and dioxin-like compounds are the classic TEF example (US EPA 2010).

Distinguishing characteristics of TEFs and RPFs are:

- RPFs are a generalized case while TEFs are a specialized case/application of the RPF approach; RPFs may be limited to specific health endpoints, while TEFs apply to all health endpoints associated with that group of chemicals;
- RPFs may be limited to specific exposure routes while TEFs apply to all exposure routes;
- RPFs may be limited to specific exposure durations while TEFs apply to all exposure durations;
- RPFs may be based on lower quality data and fewer data points with less certainty about the mode of action relative to TEFs, while TEFs are for those situations with high quality and abundant data with considerable certainty about the mode of action underlying the toxic effects.

In effect, TEFs are only applicable when there is confidence that a singular biochemical pathway, shared by all of the chemicals included in the evaluation, is the key event leading to downstream toxic events (e.g., dioxin and dioxin-like chemicals binding to the aryl hydrocarbon receptor).

Component-Based Approaches Based on Response Addition

Response addition (also referred to as independent joint action) was recommended in EPA guidelines for mixtures risk assessments as the default component-based approach to estimate the toxicity of mixtures containing chemicals with dissimilar mechanisms of action (US EPA 2000). Response addition is based on probability theory and is calculated using the equation:

$$R_{mixture} = 1 - \prod_{i=1}^{n} (1 - R_i)$$

Where

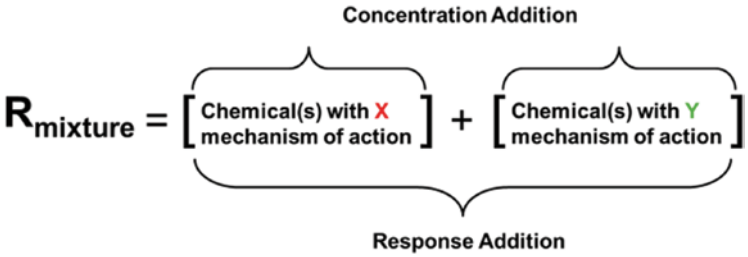
$R_{mixture}$ = the calculated response of the mixture
R_i = the known response of chemical "i" for 1 through n chemicals

There has been significant discussion in the literature regarding whether or not multiple chemicals can actually act independently in complex biological systems due to the interconnected nature of signaling pathways. Determining which combinations of chemicals and biological targets result in joint effects that can be predicted using response addition remains an active area of research.

Case study: Response additivity of dissimilar chemicals in a bacterial assay (Backhaus et al. 2000). Backhaus et al. (2000) were interested in asking if chemicals with distinctly different mechanisms of action would act in a response additive manner when present in a mixture. To do this, they selected thirteen chemicals that were toxic to the bacteria, *Vibrio fischeri*, via strictly different mechanisms. They assessed the chemicals individually to characterize the dose-response relationship for each chemical. The response addition equation (see above) was used to predict the mixture effects at a series of mixture concentrations. Next, the authors assessed the toxicity of two different mixtures. The reported findings indicate that the response addition predictions provided a good fit to the observed data, while the alternative model of concentration addition over-predicted the response of the mixture. It is notable, however, that the concentration addition model over-estimation was within a factor of three for the concentration of the mixture eliciting a 50% effect.

Integrated Addition

Integrated addition represents a combination of dose addition and response addition designed to estimate the toxicity of mixtures containing some chemicals with similar mechanisms of action as well as chemicals with different mechanisms of action (Altenburger et al. 2005; Rider and LeBlanc 2012; Teuschler et al. 2004). According to integrated addition, chemicals with the same mechanism of action are grouped together. Within each mechanism-based group, a dose addition model (see specific dose addition methods above) is used to predict the total response expected from the group. Next, the mechanism-based groups are combined using the response addition model. The equation for integrated addition is:



Where

$R_{mixture}$ is the predicted response of the mixture

In practice, the predictions based on integrated addition generally fall between those of dose addition and response addition (Olmstead and LeBlanc 2005).

Chemical and Nonchemical Cumulative Risk Assessment

People are not exposed exclusively to single chemicals or even mixtures of chemicals, but a *complex combination of chemical and nonchemical stressors*. Nonchemical stressors can elicit toxicity or modify the toxicity of chemical stressors. Therefore, it is important to consider whether and how to incorporate nonchemical stressors into cumulative risk assessment. Nonchemical stressors include biological, physical, and psychosocial factors that have the potential to disrupt normal function and lead to negative health outcomes. They can be complex and difficult to categorize. For example, sleep deprivation and noise are two nonchemical stressors that could plausibly be categorized as physical or psychosocial stressors. Therefore, the descriptions and examples here are simplified for ease of understanding and are subject to interpretation. Physical stressors act directly on biological structures or systems to disrupt function and elicit disease. Physical stressors can include: disease states (e.g., asthma, metabolic disorders), biological agents (e.g., bacteria, viruses), radiation, heat, noise, vibration, starvation, etc. Psychosocial stressors are indirect stressors in that the physical effects are secondary to the perception of the stressor. For example, low socioeconomic status (SES) does not act directly on biological systems, but does have real health implications.

Risk Assessment Approaches for Combined Consideration of Chemical and Nonchemical Stressors

Although there is increasing recognition of the need to include both chemical and nonchemical stressors in cumulative risk assessments, established methods and data on interactions among stressors are inadequate (Sexton 2012). Therefore, the approaches described below are proposed as general framework options, but do not represent standard, quantitative methods with a history of use.

Adding Nonchemical Stressors to Established Cumulative Risk Assessment Frameworks

Nonchemical stressors can be incorporated into existing cumulative risk assessment frameworks in one of two ways:

- As additional stressors within the cumulative risk assessment framework. This would require dose-response toxicity data for the nonchemical stressor, which could then be incorporated into either the HI or RPF approaches described above. These kinds of data are extremely rare for nonchemical stressors.
- As modifiers of chemical-induced toxicities. For example, assume data are available showing that a nonchemical stressor (e.g. noise) enhances the toxicity of a chemical stressor (e.g., carbon monoxide). A modifying factor could then be incorporated to decrease the AL of the chemical stressor when a risk assessment is being performed in a situation where both the chemical and nonchemical stressor

are present (e.g., occupational setting where both noise and carbon monoxide are risk factors) (Rider et al. 2012).

Community-Based Risk Assessment (CBRA)

This approach uses a particular community as a starting place and includes an evaluation of the specific chemical and non-chemical stressors within the community. The CBRA process can be initiated by the community itself, government agencies charged with evaluating public health concerns, or academic researchers. A defining characteristic is that CBRAs involve community engagement, often using a community-based participatory research framework (Israel et al. 1998; O'Fallon and Dearry 2002).

Case Study: Crow Reservation Community Based Risk Assessment of Water Contaminants (Cummins et al. 2010) Members of the Apsaálooke (Crow) tribe in Montana were concerned about deteriorating water quality in local water sources as well as perceived health disparities on the reservation, as compared with the general population (e.g., cancer clusters, gastrointestinal illness). Tribal community members partnered with a local academic, who engaged the Indian Country Environmental Assessment Program. This partnership led to an environmental health assessment, which identified water quality as the most important environmental health issue on the reservation. This effort resulted in formation of the Crow Environmental Health Steering Committee and subsequently, The Crow Water Project. Science majors from the local tribal college (Little Big Horn College) collected data on water quality and documented community concerns about water quality. These data were used in grant applications to fund interventions to improve water and wastewater infrastructure. Community surveys and monitoring of well water for pathogens and contaminants were also initiated to get a more accurate picture of exposure and health issues in the community. Efforts such as these help the community and government agencies make more informed decisions about where to focus attention and how to invest limited resources to have the greatest impact on public health.

Disease-Based Risk Assessment

According to this framework, a disease of interest is first identified and is then followed by evaluation of the potential mechanisms leading to the disease and the chemical and non-chemical stressors that could lead to the disease. Specific tools for identifying and quantifying relevant stressors and incorporating interactions among stressors are not prescribed. Instead, this approach represents a conceptual framework useful for deciding which stressors to include in a cumulative risk assessment. Once stressors are identified and characterized with respect to exposure and toxicity, they could be combined using a HI approach.

Case study: Cardiovascular Disease The figure below (Fig. 6.4) is an example of a disease-based approach for identifying factors that contribute to the development of cardiovascular disease and could be included in a cumulative risk assessment.

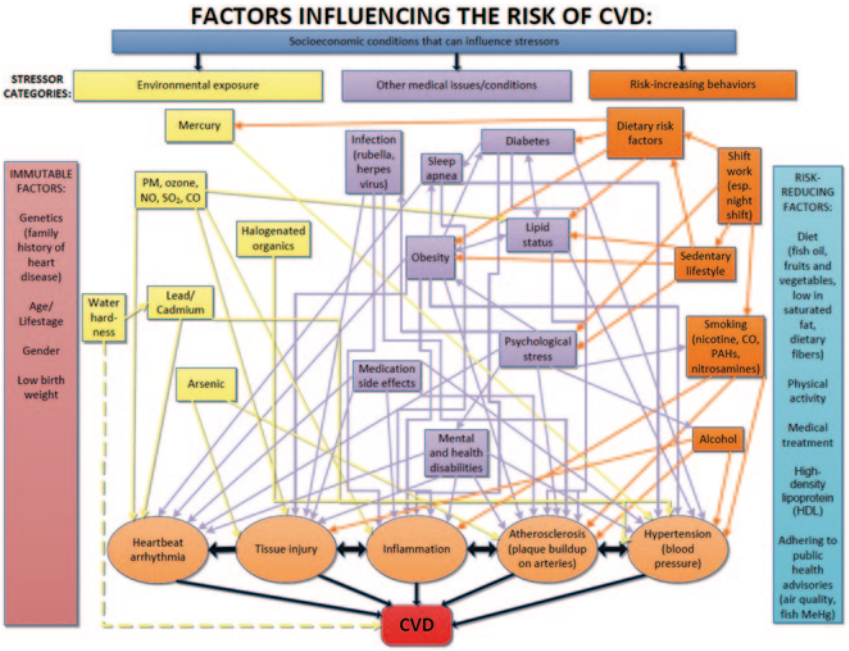


Fig. 6.4 Schematic of identified chemical and physical factors influencing the risk of Cardiovascular Disease (CVD). Prepared by C. Menzie and R. Kashuba for the EPA Workshop on Methods to Integrate Chemical and Non-Chemical Stressors in Cumulative Risk Assessment (CRA) (November 26–27, 2012). (US EPA in review)

Unsolved Problems and Emerging Issues

Risk assessment methods and techniques have evolved since the publication of EPA's risk assessment guidelines for chemical mixtures in 1986 (US EPA 1986). However, unsolved problems remain. With regard to complex mixtures, areas requiring research attention include: how to accurately account for or estimate the impact of the unidentified fraction of the mixture mass (as mentioned under Whole Mixture Techniques, substantial amounts of the mixture mass typically consists of unidentified chemicals); development of methods to determine when mixtures with insufficient data are sufficiently similar to a mixture with available data so that the mixture with sufficient data may serve as a reference mixture for the mixture with insufficient data; methods to discern the contribution to the toxicity of a complex mixture of either individual chemicals or groups of chemicals contained in the mixture, accounting for the contribution of the unidentified fraction; and, how to accurately account for changes in mixture composition over time due to such factors as environmental degradation and/or transformation and differential rates of transport through the relevant environmental media by the chemicals contained in the mixture.

With regard to defined mixtures, unsolved problems or problems for which only partial solutions have been achieved include: unbiased methods for determining

which chemical and nonchemical stressors should be included in component-based risk assessments; the extent to which the assumptions inherent in component-based risk assessment methodologies have been examined experimentally; methods to discern those component chemicals responsible for the majority of mixture toxicity; accounting for the impact of nonadditivity when present; how to effectively use high through put data streams and 'omics' data (e.g., genomics, metabolomics, proteomics) in chemical mixtures toxicology and risk assessment; the need to understand how nonadditive interactions in one target organ affect health risk in other target organs; and, the impact of additivity (either dose or response) on health risk.

The need to develop risk assessment methods for the joint health impacts of combinations of chemical and nonchemical stressors cannot be classified as an emerging issue as the need for chemical and nonchemical cumulative risk assessment is now well recognized. However, the methods, techniques and approaches with which to conduct such assessments are not yet available, so quantitative risk assessment approaches for chemical and nonchemical stressors are a clear emerging issue. Other emerging issues for cumulative risk assessment are: how to incorporate disparities in access to goods and services (e.g., health care, nutrition, income/poverty, social stress, green space, ecosystem services, lifestyle choices) that may act as buffers and decrease or mitigate the severity of the effects of exposure to either chemical mixtures or combined chemical and nonchemical stressors; and, how to effectively manage risk remediation when one or more contributors to the combined risk of chemical and nonchemical stressors may be within the domain of multiple agencies or not covered by an agency at all.

Chapter Summary

In performing cumulative risk assessments, it is critical to first evaluate the availability and quality of data. Although whole mixtures approaches are favored because they require fewer assumptions and extrapolations than component-based approaches, they are rarely used in practice due to the high data requirements and lack of established methods for determining sufficient similarity. Component-based risk assessment approaches can be based upon the concept of dose addition or response addition. In characterizing interactions (i.e., greater than additive, less than additive) among mixture constituents, it is critical to clearly identify the underlying assumption (dose addition or response addition).

The two most frequently applied methods for component-based cumulative risk assessment, the Hazard Index (HI) and the Relative Potency Factor (RPF) approaches, are based on the concept of dose addition. In both cases, the product of the cumulative risk assessment is an estimate of the risk associated with exposure to a mixture. In the HI scenario, values decreasing 1 to 0 are considered to be less concerning, while values increasing from 1 indicate increased concern for potential health risk. The HI can also be modified using interaction terms (Interaction-Weighted HI) or by including values specific to a target organ (Target Organ Toxicity HI). In the RPF scenario, the resulting product is a predicted response

of the mixture based on the cumulative dose of the mixture expressed in terms of an index chemical. Although nonchemical stressors are recognized as important contributors to cumulative risk, methods for quantitatively incorporating them into cumulative risk assessments remain theoretical.

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Chapter 7
Use of Epidemiology in Risk Assessment

Martin D. Barrie and Gregory Nichols

Abstract Epidemiology is a branch of public health that evaluates relationships between exposures and adverse outcomes within specific populations. One of the critical areas of environmental and occupational epidemiology is the assessment and evaluation of potential causal associations between exposures of interest and identified adverse outcomes. Epidemiologists rely on a variety of tools to assess this relationship, and epidemiology has very useful applications in the risk assessment process. The integrative use of epidemiology in the risk assessment process not only assists in identifying and evaluating hazards, but it can also be used to better characterize situations and conditions for reducing, eliminating or mitigating the burden of disease through controlling hazardous exposures. Epidemiology (in conjunction with risk assessment) can play an integral role in the formulation of health policy and regulation.

Keywords Epidemiology · Bradford Hill · Association · Study designs · Methods · Integration

Student Learning Objectives

After reviewing this chapter, you should be able to:

- Describe the study designs used in epidemiology and their limitations
- Describe the relationship between exposure-response and dose-response

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- Explain and distinguish the concepts of association and causation
- Describe the Bradford Hill considerations and their limitations in causation
- Explain the application, integration and limitations of epidemiology in risk assessment

Epidemiology Overview

Brief History

Epidemiology is the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems (Last 2001). It is often considered the foundation of public health, as it plays a crucial role in developing methods for disease prevention, identifying efficient forms of medical treatment, and formulating health policy. There are five core objectives of epidemiology (Gordis 2009):

1. To identify the cause and associated risk factors of a disease
2. To determine the extent of illness or injury in a population
3. To study the formation and progression of a disease
4. To evaluate preventive and treatment measures
5. To provide the basis for developing health-related public policy

The beginnings of modern epidemiology started with John Snow (1813–1858). Snow was a nineteenth century physician who lived and worked in London. During the early and middle part of the century, London was subject to multiple outbreaks of cholera. Building on his work from studying previous cholera outbreaks, Snow investigated the 1854 epidemic and eventually tracked down the Broad Street water pump as the source of the infection. He recommended that the pump be shut down. The spread of the epidemic slowed after the pump handle was removed, although it is commonly believe that the outbreak was waning and naturally would have died out anyway. Nonetheless, his investigation and subsequent action was an important turning point in the development of modern epidemiology and its application to protecting the public's health.

Building on the work of Snow and others, epidemiologists were able to make recommendations for disease prevention measures that greatly improved the health of people. In fact, people began to live so much longer because they were not dying of infectious diseases anymore, that the emergence of a chronic disease epidemic became evident. Chronic diseases (e.g., cancer, diabetes, and heart disease) are not caused by pathogens but are, in fact, caused by multiple factors (e.g., family history, cholesterol, chemical exposures, smoking, etc.) and affect everyone differently. This transformation from infectious disease to non-infectious (chronic disease) is known as the *epidemiologic shift*.

Epidemiologists in the mid-twentieth century began studying chronic disease in order to find some possible explanations of cause. Some important findings were strengthening the association between cigarette smoking and lung cancer (based on the work of Austin Bradford-Hill and Sir Richard Doll) and identifying the risk factors for cardiovascular disease (mainly due to the Framingham Heart Study begun by Thomas Dawber in 1948).

A new set of problems was identified in the late twentieth century and stretching into the early twenty-first century. These diseases were mainly the result of mental health and social conditions. Epidemiologists still struggle with developing theories to explain chronic diseases and continue preventive measures for controlling infectious diseases, but figuring out risk factors for suicide, drug addiction, alcoholism, and domestic violence has become a new challenge. As the field of epidemiology continues to be refined, a new set of tools is being developed and continues to build on the foundation set by previous epidemiologists.

Specializations

Epidemiology covers a wide range of topics, and like most sciences, epidemiology is divided into different branches. Some common epidemiologic specialties are:

Cancer Epidemiology—The study of the factors affecting the development and evaluation and treatment of cancer.

Clinical Epidemiology—The application of epidemiologic concepts and methods to problems (diagnostic, therapeutic, and preventive) encountered in the delivery of health care to patients.

Environmental Epidemiology—The branch of epidemiology that studies environmental factors associated with illnesses and deaths.

Infectious Disease Epidemiology—The branch of epidemiology that studies epidemics and the factors involved in the transmission of infectious agents in populations.

Molecular Epidemiology—The branch of epidemiology that studies the etiology, distribution, and prevention of disease at the molecular level (typically focused on identifying pathways associated with genes and environmental risk factors).

Occupational Epidemiology—This branch of epidemiology involves the application of epidemiologic methods to worker populations and looks at exposures to chemical, biological, or physical agents to determine if the exposures result in the risk of disease.

Pharmacoepidemiology—The study of the use and effects of drugs in large numbers of people.

Radiation Epidemiology—The study of the effects of radiation, ranging from cellular change to cancer, in human populations.

Social Epidemiology—The branch of epidemiology that studies the specific features of societal conditions in order to explain patterns of health in a population.

Veterinary Epidemiology—Veterinary epidemiology investigates diseases in animal populations to describe how animal welfare is affected by the interaction of different factors in order to reduce the frequency of disease occurrence.

Elements of Epidemiologic Studies

Although there are many different types of study designs, there are several fundamental elements as described by Blumenthal et al. (2001) that transcend all types of epidemiological studies:

1. Formulation of the study question or hypothesis
2. Selection of study populations and study samples
3. Selection of indicators of exposure
4. Measurement of exposure and disease
5. Analysis of the relationship between exposure and disease
6. Evaluation of the role of bias
7. Evaluation of the role of chance

Although volumes of work exist regarding the elements of epidemiological studies, a brief description of each study element follows below. While not intended to be comprehensive, these descriptions form the basis for epidemiology and risk assessment.

Formulation of the Study Question or Hypothesis The study question must be formulated in such a way that it can be tested using statistical methods. Fundamentally, data collected from the question is used to test the null hypothesis, which implies that there is no relationship between an exposure and a disease outcome (Blumenthal 2001).

Selection of Study Populations and Study Samples A study population exposed to the factor of interest and a control population not exposed to the factor of interest need to be selected in order to make statistical comparisons (Blumenthal 2001). Basic techniques such as matching, in which controls are selected so that they are similar to cases in certain characteristics, like age, gender, and occupation, but not for the characteristic being studied (Gordis 2009), are used to ensure that bias and error are reduced.

Selection of Indicators of Exposure Selecting an adequate and reliable test to evaluate the exposure of interest must be determined prior to the beginning of the study. Various methods for characterizing and measuring the exposure of interest may exist (e.g., medical records, biomarkers, and environmental sampling) and investigators must ensure that the most valid and reasonable method is used.

Measurement of Exposure and Disease This particular element covers the assignment of individuals to either exposed or non-exposed groups or to cases or controls. It is a very important step in the process, as misclassification, which is an erroneous classification of an individual, a characteristic, or a measured value into a category other than where it belongs, can have major implications in evaluating the relationship between exposure and disease and assigning risk to the study population.

Analysis of the Relationship Between Exposure and Disease In the study population, measurements of exposure and disease status must be made while minimizing the various types of error that can occur. Simple measures of disease frequency, such as prevalence (the number of existing cases of disease in a population at a specific point in time) and incidence (the number of new cases of disease in a population within a given period of time) are often used in epidemiology to estimate burden of disease. Measures of risk such as relative risk (RR), which is a comparison of the risk of disease in an exposed group versus the risk of disease in an unexposed group, or odds ratio (OR), an approximation of risk as the odds of exposure among cases of disease to the odds of exposure among the control group, are typically used to evaluate potential associations in most studies. Sometimes, higher level statistical analysis, such as linear regression or linear correlation, which measure the strength of bivariate association or predicts estimate for values are needed (Kuzma 2005).

Evaluation of the Role of Bias Bias is systematic error that may results in the incorrect estimate of the association between exposure and disease. Many different types of bias exist, but some of the most common are:

- Recall bias—error due to a study participant incorrectly remembering previous events.
- Selection bias—error that can arise from differences that exist among those who participate in a study versus those who do not participate (an all-volunteer study is a classic example of selection bias).
- Information bias—a flaw in reporting data so that there is a difference between the quality of information collected from various groups (Last 2001).

One of the greatest challenges in any study is to protect against confounding, which is not an error in the study, but is a real phenomenon that is identified and must be understood (Gordis 2009). A confounding factor must satisfy three conditions: (1) it must be associated with the disease (but cannot be an effect of the disease), (2) it must be associated with the exposure, and (3) it must not be an effect of the exposure (Rothman 2002). A fallacious conclusion concerning the confounding factor may lead to an untruthful belief that there is a causal relationship between the confounding factor and some other factor in the study. In order to control for this effect, tools such as matching and stratification, separating the data into groups (e.g., by age, location, gender, and smoking history) are used.

Evaluation of the Role of Chance Evaluating chance involves two components: hypothesis testing and the estimation of a confidence interval, or the range in which the true estimate of the effect is likely to lie. Hypothesis testing involves calculating a p-value to measure the statistical significance of the results and to explain the

observed effects. Estimating a confidence internal reflects the precision of a point estimate of effect, and is reflected in measures of risk (Blumenthal 2001).

Study Designs

One of the most important considerations in epidemiology is the study design. The way a study is framed determines what kind of information will be collected, how that information will be used, and what types of measurements will be calculated. Epidemiologic studies can be divided into different groups (see Gordis 2009; Rothman 2002), but three types predominate (Blumenthal 2001):

- Descriptive
- Analytic (observational)
- Experimental (interventional)

Descriptive studies yield outcomes related to morbidity, mortality, prevalence, and incidence. They are often used to generate hypothesis. Analytic (observational) studies are studies in which researchers are only collecting and analyzing data; there is no interference with study subjects. These are the most common types of studies used by epidemiologists. Experimental studies are not passive, and they involve the direct manipulation of exposures and subjects. Clinical trials used to develop and test prescription medication are the best example of experimental studies.

Epidemiologists conduct studies in order to calculate the probability that certain conditions (exposures) could be associated with death or disease (outcome) in a particular population. This relationship between exposure and outcome is the basis of calculating risk, which is the likelihood that a particular event will occur. Different study designs determine how risk is calculated and interpreted.

Ecological Studies An ecological study is a type of study in which the units of analysis are populations or groups of people, rather than individuals, at one point in time. A researcher will choose the data (for example, average cholesterol levels, heart attack deaths, or cancer cases) for several geographic areas (e.g., cities, states, or countries). Information can be collected from multiple data sources: registries, census records, death certificates, or medical records. For ecological studies, correlation (r) is a good indicator of how the data sets are related. The value of r is always between -1 and 1. The closer r is to 0, the less likely that a significant relationship between the data exist. As a value approaches -1 or 1, the more likely a significant relationship exists between the data. A key point to consider is that correlation does not imply causation because the measurement of the two variables is not made in individuals.

One major drawback to ecological studies is that the results can lead to what is known as the *ecologic fallacy*. This means that the results of a study are often assumed to represent all individuals of that particular study area, which may not be correct. In addition, ecological studies compare characteristics of geographic boundaries only; therefore, individuals cannot be studied.

Cross-sectional Studies Cross-sectional studies examine the relationship between diseases and exposures in a population at one specific point in time. When designing a cross-sectional study, a researcher defines a population and determines the presence or absence of exposure and disease for each individual. There will be four outcomes in this population:

a. Those who were exposed and have the disease
b. Those who were exposed and do not have the disease
c. Those who were not exposed and have the disease
d. Those who were not exposed and do not have the disease

Cross-sectional studies are sometimes called prevalence studies because in the process of testing individuals, we can measure the number of people who have the disease at a specific point in time (prevalence).

Cross-sectional studies are popular because they are convenient and relatively easy to perform. They can be done in a short period of time at a relatively low cost. Also, cross-sectional studies can be used to make generalizations of the population from which the sample was drawn. The major drawback of a cross-sectional study is that it is often difficult, if not impossible, to know if the disease or the exposure came first, since both are measured in an individual at the same time.

Case-control Studies A type of analytical study, the case-control study compares people with a certain disease (cases) with people who do not have that same disease (controls). In case-control studies, we cannot calculate incidence since we already know who has the disease; therefore, we estimate risk through the odds ratio.

Typically, case-control studies begin with the selection of cases. These can come from hospitals, clinics, or registries. Investigators must be careful when selecting cases, as the focus should be in trying to select subjects that would represent the general population as much as possible. Controls can be selected from just about anywhere. They can be individuals living in the same communities as the hospitals where cases were selected or controls can be other admitted patients who do not have the same disease as cases.

It is important that the control group has similar age, ethnic, and gender distributions as the cases. If cases and controls are not similar, it can be difficult to know if the exposure of interest has an effect on the development of disease or if one of the other characteristics of the groups is responsible for the disease. For this reason, controls are often matched to cases. Case-control studies are useful for studying rare diseases since we do not have to wait for the disease to develop as cases are selected at the beginning of the study.

Cohort Studies The most robust type of analytical study is the cohort study. Cohort studies follow groups of people (cohorts) through time, either forward or backward, and compare an exposed group with an unexposed group. Researchers look at the cohorts and determine if disease has developed in the groups and then compare how much disease has developed in the exposed group versus the unexposed group.

A study that starts with a reference population, some of whom have the exposure relevant to the study and others who do not, is known as a prospective cohort study.

Both groups, at the outset of the study, should be free of the condition (disease) under consideration. The entire disease-free cohort will be followed through time to determine which cohort members develop the disease.

Retrospective cohort studies are similar to prospective cohort studies, but the follow-up period has been completed. In other words, we typically define a population and then go back in time and reconstruct the exposure for each individual. This is the type of research design that is typically used to study industrial populations. Each individual is healthy enough to be hired, so the researcher knows that the cohort is generally disease free at the beginning of the study. Historical records can be assembled to reconstruct the exposure patterns for each individual.

The essential element in the design of a cohort study is the comparison of the outcome (disease) between the exposed and non-exposed groups. Relative risk is typically used to evaluate potential associations in cohort studies. The stronger the relative risk, the greater the association between exposure and disease. Relative risk is used in cohort studies, because we compare the incidence of disease in the exposed group with the incidence of disease in the non-exposed group. Only cohort studies where we start with a disease-free population can show us incidence.

Relative risk is generally interpreted using the following guidelines (Gordis 2009):

- If RR < 1 then risk in the exposed group is less than the risk in the non-exposed group (negative association, possibly protective)
- If RR = 1 then risk in the exposed group is equal to the risk in the non-exposed (there is no association)
- If RR > 1 then risk in the exposed group is greater than the risk in the non-exposed group (positive association, possibly causal)

Epidemiologists like to use cohort studies when there is interest in establishing causation because the exposure is known to precede disease. Prospective cohort studies are used to directly measure the relative risk of developing a disease for people in different exposure categories. One major benefit of prospective cohort studies is that they can reveal multiple diseases related to the same risk factor.

Experimental Studies Experimental or interventional studies are conducted to allow safety and efficacy data to be collected for health interventions. These studies considered the ideal study design for scientific strength since they utilize a principle called randomization. Randomization significantly reduces bias by ensuring that the study and sample selection process can be completely reproducible by anyone at any time. Randomly assigning individuals to categories in experimental studies also reduces bias in that investigators cannot anticipate that the results may be based on any particular pattern.

As much as experimental trials are desirable for their rigorous scientific conduct, they are extremely expensive to conduct. Often, these studies are split into multiple phases to test increasing strengths of interventions with different sample sizes. Additionally, these types of studies are used to measure the effectiveness of treatment; they are not used to measure risk of developing disease or the association between

exposure and disease. Experimental studies are not typically used in environmental studies as they pose some serious ethical and legal issues.

Evaluating Epidemiologic Studies Each study will have its own data, target population, exposure (s), and outcome (s), but all studies ask questions about three things (MacMahon 1970):

1. Person—Information including age, gender, ethnicity, marital status, occupation, and socioeconomic status.
2. Place—Considers data from international comparisons, variation within countries, urban-rural comparisons, and local distributions.
3. Time—Tracks data collected on the basis of calendar time, cyclic fluctuations, and clustering in time.

Questions about person, place, and time provide answers for who is being studied, where are they being studied, and in what period of time are they being studied. In addition, we may also ask: what is being studied and what is the outcome of interest, why is a particular effect happening (like an increase in disease cases or deaths), and how is it happening (vector-borne, person-to-person contact, genetic transmission, etc.).

Evaluating the outcome of a study is an important part of epidemiologic research and analysis. To determine the nature of the study consider asking these questions:

- What is the exposure of interest, and how was it measured?
- What is the outcome of interest, and how was it measured?
- What is the population, and how was it defined?
- What is the comparison group, and is it appropriate?
- What measures were used to evaluate the relationship between an exposure and an outcome?

While these questions may not answer everything, they are a good starting point in terms of gathering information about the validity of the study.

Exposure-Response

The exposure-response relationship is one of the essential principles of epidemiology. If a causal relationship exists, then the risk of disease will increase as the level of the exposure increases. Epidemiologists typically do not use "dose-response" as this is a toxicological term related to body burden. If an exposure-response relationship does exist, there is strong evidence for a causal relationship, but the absence of an exposure-response relationship does not rule out a causal association (Gordis 2009). A typical exposure-response relationship exists with cigarette smoking and lung cancer. The amount of cigarettes smoked per day is directly related to the risk of acquiring lung cancer.

Case-control and cohort studies provide the best methods of estimating the exposure-response relationship. Through risk ratios (RR and OR), a strength of associa-

tion can be measured between a particular exposure and corresponding disease. A rough guide of risk estimates is as follows:

- 1.0 = None
- > 1.0– < 1.5 = Weak
- 1.5–3.0 = Moderate
- 3.1–10.0 = Strong
- > 10.0 = Very strong

One of the most telling characteristics of an exposure-response relationship is what happens when the exposure is removed or minimized. If a causal relationship does exist, then the cessation of the exposure should lead to a decline in the risk or acquiring the disease though to be caused by that exposure (Gordis 2009). In some cases, the biologic damage may be too sever after a period time and hence, irreversible (such as the case with smoking and chronic obstructive pulmonary disease), but the elimination of the exposure, will most likely slow the progression of illness.

Association vs. Causation

The ultimate goal of epidemiology is to provide enough data and information to determine if a statistical relationship exists between an exposure and an outcome. In health-related studies, epidemiologists must distinguish between association and causation. An exposure can have a causal relationship with a disease (e.g., smoking can cause lung cancer) or an exposure can be associated with a disease (smoking is associated with alcoholism). Because an exposure and a disease are associated does not mean that there is a causal relationship. Epidemiologists cannot prove causation with 100 % certainty, but they often provide enough scientific fact to meet certain criteria to strengthen the argument that a causal pathway could exist.

Association The search for a causal pathway begins with a common chain of events. Observations of natural phenomenon might lead to questions about if an exposure is related to a disease. Data are collected using some of the techniques mentioned previously, and then case-control or cohort studies are typically conducted to determine if a statistical relationship exists. When a statistical association is found, investigators should ask six primary questions (Friedman 2004):

1. Could this be due to chance?
2. Could this be due to bias?
3. Could this be due to confounding?
4. Is the association the same in all subgroups, or does it vary in relation to some other characteristic(s)?
5. To whom does this apply?
6. Does the association represent a cause and effect relationship?

If the association appears to be real, then epidemiologists take some further steps to test whether or not the relationship is true.

Types of Causal Relationships Once it is determined that an association exists between an exposure and an outcome, the epidemiologist will try to piece together an explanation. Sometimes, this can be a very complicated issue, considering the complex nature of biologic variability. Causal pathways are either direct or indirect. In the direct route, a factor causes disease without any intermediate steps. In the indirect route, one or more intermediate steps take place between the exposure and the development of disease.

When determining types of causal relationships, epidemiologists look at factors and try to determine if they are necessary, sufficient, neither, or both. A necessary cause must always precede an event, and a sufficient cause is good enough to produce an event, but it does not mean that the event will always as other factors could produce the same result. There are four possibilities, given necessary and sufficient causes (Gordis 2009):

1. *Necessary and sufficient*—Without the factor, the disease never develops, and in the presence of the factor, the disease always develops (this rarely occurs, if ever)
2. *Necessary, but not sufficient*—The factor must be present to cause disease, but other factors must be involved to complete the process (e.g., the tubercle bacillus is necessary to cause TB, but not everyone exposed to the bacillus will develop TB)
3. *Sufficient, but not necessary*—The factor alone can produce disease, but other factors can produce the same disease as well (e.g., radiation exposure and benzene exposure both cause leukemia, but both factors together are not required to cause leukemia; also, not everyone exposed to radiation or benzene will develop leukemia)
4. *Neither sufficient nor necessary*—A factor by itself cannot cause disease (e.g., chronic diseases most likely operate this way)

Sir Bradford Hill Considerations and Limitations

It is difficult to prove that a causal relationship exists with certainty, but a set of guidelines (Hill 1965) has been suggested by Sir Austin Bradford Hill to aid investigators in determining the likelihood that a causal relationship does exist. The "Hill criteria," as they are often described, are listed in Table 7.1, along with some limitations relating to each of them. The application of the Hill criteria in a weight of the evidence approach to causal inference can be explored by reviewing Swaen and Amelsvoort (2009).

Table 7.1 Bradford Hill criteria and suggested limitations

Criterion	Definition (Hill 1965)	Limitation (Rothman 2002)
Strength	The strength of the association is measured by the relative risk or odds ratio: the greater the risk, the greater the support for causality. The stronger the association, the more likely it is that the relation of "A" to "B" is causal	Strength depends on the prevalence of other causes and, thus, is not a biologic characteristic; could be confounded
Consistency	A possible causal relationship between exposure and disease should match the data of other similar observations for the same exposure and disease pair in studies replicated in different settings and populations using different methods	Exceptions to consistency are often identified with the additional data and information (hindsight)
Specificity	Specificity refers to the fact that a certain exposure is responsible for only one disease. If one exposure does indeed lead to only one cause, the case for causality is strengthened (as normally happens with infectious diseases). However, the absence of specificity does not negate a causal relationship. Outcomes are likely to have multiple factors of influence and it is unlikely to find a one-to-one relationship between multiple factors	A cause can have many effects
Temporality	If an exposure is thought to cause disease, then it is necessary for the exposure to occur before the onset of disease. This is the only absolutely essential criteria	It may be difficult to establish the temporal sequence between cause and effect
Biologic Gradient (Dose-Response)	If an association exists between an exposure and disease, then we would expect to see a higher incidence of disease in individuals that are more exposed. The presence of a dose-response relationship is strong evidence for causation. However, as with *Specificity,* the absence of a dose-response relationship does not rule out a causal relationship. A threshold may exist above which a relationship may develop	Could be confounded; threshold phenomena would not show a progressive relation
Plausibility	Any causal relationship must be consistent with accepted views of the biological process. In other words, some reasonable and rational process by which an exposure could cause disease	Too subjective
Coherence	The association should be compatible with existing theory and knowledge. In other words, it is necessary to evaluate claims of causality within the context of current state of knowledge (within a given field and in related fields). This principle refers to the fact that any theory about a particular exposure/disease relationship should be compatible with generally known facts about the disease	How does it differ from consistency or plausibility?

Table 7.1 (continued)

Criterion	Definition (Hill 1965)	Limitation (Rothman 2002)
Experimental Evidence	If a true causal relationship exists between an exposure and disease, then the disease rate should go down if the exposure is lessened or removed. Experimental evidence is one of the strongest criterions for establishing causality.	Not always available
Analogy	The extent to which other possible explanations have been taken into account and the extent to which such explanations have been ruled out	Analogies abound

Epidemiology and Risk Assessment Application

The application and methodologic issues associated with the use of epidemiology in the risk assessment process have been reported and discussed by several investigators (Samet et al. 1998; Nurminen et al. 1999; Blumenthal et al. 2001; Nachman et al. 2011). In the integrative application of epidemiology into the risk assessment process, a useful modeled approach described by Nurminen et al. (1999) would correlate the following: Hazard Identification—Descriptive Epidemiology; Exposure Assessment—Molecular Epidemiology; Dose-Response Assessment—Exposure-Response; and Risk Characterization—Intervention Epidemiology.

Hazard identification is "inherently integrative" and involves the compilation of all relevant lines of scientific evidence (Samet 1998). It can be argued that the application of epidemiology is essential in the risk assessment process in that epidemiology provides information on humans. Although limited to populations, epidemiologic investigations to provide population-based strengths of association between specific exposures and specified adverse health outcomes. The epidemiologic evidence can be as broad as descriptive population-based evidence or as specific as increased risk ratios associated with exposures on the genetic level.

Descriptive epidemiology makes use of all available data from various implemented study designs and analysis that allows for the identification and characterization of morbidity and mortality rates, describes and characterizes demographic variability, identifies high-risk groups, and provides information on person, time and place differences and variability. Geographical investigations also allow the characterization of environmental exposures and confounders of smoking and diet on exposure-disease associations. For example, disease clusters may be identified in specific locations without an obvious putative source of association. The observed increased risk of mesothelioma among certain Turkish residents was observed as a cluster, with later determination of asbestos containing minerals used in whitewash for homes. Studies on industrial pollution from localized point sources can also be characterized for putative disease association, evaluating and stratifying exposure and disease type. Residence location and proximity to waste sites and incinerators can also help evaluate exposure-disease relationships. As epidemiologic data help

characterize human exposure-response relationships, this data is useful in identifying the hazards. A good example would be the use of a hazard surveillance system to identify and monitor hazards for disease risk. Another example would be compensation systems that compensated for various exposures. The results of molecular epidemiologic investigations can also be used to assess exposure; for example, where the frequency and distribution of particular genetic markers or aberrations are identified among various occupational exposures (see discussion on benzene below).

Epidemiology also provides information that supports hazard identification and characterization. There are, however, some limitations or weaknesses with the use of epidemiologic data in the risk assessment process. Most importantly, retrospective epidemiologic investigations rarely have accurate and reliable data on exposure. This lack of true exposure data and the need for conduction retrospective exposure assessments impacts the ability to accurately develop and assess the exposure-response relationship. Epidemiology also suffers from a lack of insight into the mechanism of the disease process, thus limiting the possible true characterization of the exposure-response relationship.

The use of epidemiologic data is also helpful in characterizing/assessing exposure assessments. The magnitude of the risk of a particular disease following an exposure is predicated on an accurate exposure assessment based on measured exposures. Unfortunately, direct quantitative data on historical exposures is often absent or marginal. In this case, qualitative and semi-quantitative epidemiology can assist by utilizing existing data and data from similarly situated populations to evaluate the magnitude of risk. Overall risk can be evaluated by looking at measures of central tendency (mean, median, and mode). Magnitudes and durations of exposure can also be characterized.

Exposure-response assessments in epidemiology are similar to dose-response in toxicology. The major difference is that epidemiology is looking at population data. However, well designed and conducted epidemiologic investigations can provide valuable insight into the nature of association between exposure and disease risk. For example, if you're able to design an investigation with stratified quantitative exposure data for evaluation of disease risk it may be possible to identify increasing trends of disease risk with each increasing stratum of exposure. The investigation could also be designed to investigate increasing disease risks associated with increasing exposure strata using the metrics of duration of exposure, magnitude of exposure, intensity of exposure, and frequency of exposure as a metrics of cumulative exposure. This stratification of exposure to evaluate risk can also be useful in determining whether there is possible a linear or threshold model at work in the population under investigation. One area to keep in mind, however, is that associated with exposure misclassification. Misclassification of exposure in the study may impact the exposure-response characterization and also may have wide variability in confidence.

Quantitative risk assessment typically relies on animal experimental data and the need to extrapolate this data to humans. Epidemiologic data derived from human investigations do not need interspecies extrapolation. Epidemiologic evaluations

of cytogenetic biomarkers can also aid in the interpreting the putative association between exposure and disease as well as aid in the characterization of the exposure-response relationships. Epidemiologic investigations also afford the benefits of being able to study the effects in heterogeneous populations and evaluate cumulative exposure. The results of epidemiologic investigations also provide insight into the viability of regulatory exposure limits, assessing whether exposure-response relationships exist at lower regulatory limits and whether they are true limits on disease risk. In establishing exposure-response relationships, epidemiologic investigations can be used in the regulatory standard setting process.

Epidemiologic findings of no effect or risk are also problematic. It is difficult to interpret these results as evidencing a true no association or whether there were issues associated with the studies or analytical methodologies. Additional potential limitations in the use of epidemiology in the risk assessment process include exposure or outcome misclassification, confounding impacts on either exposure or disease, and the impact of disease latency potentially limiting the studies ability to observe effects from exposure.

Risk characterization ties together the prior components and results of the risk assessment process and helps describe the nature and presence, or absence, of risk. Uncertainties and limitations surrounding this judgment can also be conveyed. The results of epidemiologic investigations can be helpful in characterizing populations at risk, the magnitude of the risk, overall disease burden and life expectancy, all with levels of statistical confidence. Prevention strategies can also be developed and measured from these results, for efficacy and effectiveness. Health policies (occupational and environmental) can also be developed from the data.

Benzene Example

To explore the role and use of epidemiology in the risk assessment process, benzene and its associated health risks can be used by way of example. Low-level benzene exposure will used in the example.

Descriptive Epidemiology Benzene is an aromatic hydrocarbon and is a typical component of gasoline, ranging in concentration from approximately 1 ½ percent to upwards of 5%. It is also a component of crude oils and historically has been used in inks, glues, paints, and adhesives, and in the production of rubber, plastics, chemicals, resins, dyes and explosives. The primary use of benzene today is in the manufacturing of organic chemicals, including analine, alkylbenzenes, ethylebenzene, cumene, and cyclohexane.

Benzene is a recognized carcinogen (leukemogen) by the Occupational Safety and Health Administration (OSHA), the Environmental Protection Agency (EPA), and the International Agency for Research on Cancer (IARC). At sufficient doses, benzene produces blood disorders and affects the bone marrow. Acute myelogenous leukemia (AML) has a reported association with sufficient benzene exposure, with other blood dyscrasias also being reported and suggested, including non-Hodgkin's

lymphoma, chronic lymphocytic leukemia, multiple myeloma and the myelodysplastic syndromes. A comprehensive review on the issue of hematopoietic disorders associated with benzene exposure has recently been reported (Galbraith et al. 2010), with the conclusion that only AML being clearly linked to benzene exposure.

This conclusion is in contrast to other investigations reporting associations between benzene exposure and the myelodysplastic syndromes, chronic lymphocytic leukemia, multiple myeloma, acute lymphocytic leukemia and possibly non-Hodgkin's lymphoma and chronic myelogenous leukemia (Khalade et al. 2010; Vlaanderen et al. 2012; Schnatter et al. 2012). A recent review has suggested that future investigation should focus on the biologic mechanisms for benzene-associated leukemia (Snyder 2012).

The first *IARC Monographs* to report on benzene (Volume 29, 1982) concluded that there was sufficient evidence in humans for the carcinogenicity of benzene, referencing epidemiologic cohort and case-control studies showing a statistically significant association between occupational benzene and benzene-containing solvents and leukemia. The leukemia type identified was primarily myelogenous leukemia. Later epidemiologic evidence identified an increased risk of acute non-lymphocytic leukemia (ANLL), resulting in *IARC* (*Monographs,* Volume 7, 1987) to classify benzene as a Group-1 carcinogen. The most recent *IARC Monographs* Volume 100F (2012) also concludes that there is sufficient evidence in humans for the carcinogenicity of benzene.

The issue of low-level benzene exposure and myelodysplastic syndrome risk in petroleum workers was recently evaluated and reported (Schnatter et al. 2012). Updating three nested case-control studies from Australia, Canada and the United Kingdom with new incident cases of lymphohematopoietic cancers, including acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), myelodysplastic syndromes (MDS), and myeloproliferative disorders (MPD), the investigators reported a monotonic dose-response relationship between benzene exposure and MDS (highest vs. lowest tertile, OR = 4.33, 95% CI: 1.31 to 14.3). Interestingly, there was little evidence of a dose-response relationship between reported benzene exposure and AML, the type of leukemia most associated with benzene exposure. No dose-response relationship was also observed for reported benzene exposure and CLL, CML, MPD. This investigation raises the issue of low-level benzene exposure and AML, and suggests a relationship between low-level benzene exposure and MDS.

The National Center for Environmental Assessment (NCEA) reviewed the epidemiological literature on low level benzene exposure and evaluated the available scientific literature on the use of a linear model to assess cancer risk at low level exposures (USEPA 1998), concluding that "there is not sufficient evidence to currently reject a linear dose-response curve for benzene in the low-dose region, nor is there sufficient evidence to demonstrate that benzene is, in fact, non-linear in its effects". The authors noted that while the carcinogenicity of benzene at high occupational exposures had been established, "below 40 ppm-years the shape of the dose-response curve cannot be determined on the basis of current epidemiologic data".

Molecular Epidemiology Various lines of evidence from investigations suggest that the cytogenetic effects of benzene and induction of chromosomal aberrations is likely to play an important role in the development of leukemia (Hayes et al. 2001). The mechanism of benzene-induced leukemia in humans and the implication for the risk assessment process has recently been reported by McHale et al. (2012). Included in their analysis was a mode of action approach to risk assessment and the application of toxicogenomics and the modeling of the dose-response relationship of particular biomarkers of exposure, including genetic damage as a result of hematoxicity following benzene exposure. A recent review of the use of biomonitoring data in exposure and risk assessment of benzene (Arnold et al. 2013) highlight some of the limitations with using biomarkers of exposure, including determining what the relationship is between biomarkers of exposure and subsequent adverse effects.

One of the early investigations to evaluate chromosome damage as a result of benzene exposure was conducted by Pollini and Colombi (1963) and discussed and reviewed by Zhang et al. (2002). Evaluating the bone marrow and peripheral blood lymphocytes from patients with blood disorders from severe benzene exposure, including leucopenia and aplastic anemia, they described increased findings of chromosomal structural and numerical anueploidy.

In a case-control study of 50 acute myeloid leukemia's, 17 chronic myeloid leukemia, and 19 cases of myelodysplastic syndrome patients treated in the Main Hospital of Torino, Northern Italy between October 1, 1989 and December 31, 1990, Ciccone et al. (1993) evaluated various exposures (including benzene) and the risk of chromosomal aberrations by classification under the International System for Human Cytogenetic Nomenclature. Among men exposed to benzene, petrol refined products and polycyclic aromatic hydrocarbons, there was a reported non-significant increase risk in chromosome abnormalities; OR = 1.7 (95 %CI, 0.6 to 5.5), OR = 1.9 (95 %CI, 0.5 to 7.1), and OR = 1.7 (95 %CI, 0.7 to 4.3), respectively.

Occupational chronic exposures to high concentration of benzene has also been associated with a high frequency of loss of all or part of chromosomes 5 and/or 7, as well as trisomy 8. (Stillman et al. 1997) It is thought that the benzene metabolite hydroquinone (HQ) is responsible for this chromosome 5 and/or 7 loss in benzene induced MDS/AML, and is probably due to selective cell survival after HQ exposure as opposed to HQ directly targeting chromosomes 5 or 7.

The most common cytogenetic changes in both therapy and chemically related leukemia is the loss and long (q) arm deletions of chromosomes 5 and 7 (−5, −7, del (5q) and del (7q). (Zhang et al. 1998a) In human lymphocytes, the benzene metabolites hydroquinone and 1,2,4-benzenetriol were effective in inducing changes in chromosome 5 and 7, and that chromosome 7 was particularly susceptible to aneusomy at low doses. It has been suggested that chromosomes 5 and 7 may be useful biomarkers of early biological effect for benzene exposure (Zhang et al. 1998).

In a study of 100 children with myelodysplastic syndrome, juvenile myelomonocytic leukemia, and acute myeloid leukemia, loss of chromosome 7 was observed in

75 % of those with MDS and 32 % with AML (Hasle et al. 1999). Monosomy 7 has a special role in the development of de-novo AML (Krauter et al. 1999).

Printers have also been investigated for chromosome aberrations, to evaluate their exposures to various solvents, dyes and inks, including benzene. In an investigation of 42 printers with an average of 12 years exposure to toluene and dyes, Pelclova et al. (1990) found significant differences in the number of cells with structural chromosome aberrations among those exposed when compared to controls. The difference between the two groups was significant. Cytogenetic and chromosomal damage was also investigated by Aksoy and colleagues in a study of fourteen volunteer offset printing workers (Aksoy et al. 2006). Significant chromosome aberrations were found in nonsmoking workers for all defined age groups. Among those exposed, chromatid breaks were commonly observed and to a lesser extent chromosome breaks. The authors concluded that chromosome aberration analysis, in comparison to sister chromatid exchanges, was a more sensitive end-point to evaluate environmental contaminant exposure and that chronic occupational exposures among offset printers were at an increased risk of genetic damage.

In a pilot study to investigate the relationship between occupational exposures to solvents and chromosomal abnormalities in patients with myelodysplastic syndrome, Vineis et al. (1990) identified 57 patients with newly diagnosed acute or chronic leukemia from October 1988 to June 1989 in Torino, Italy. Eleven of the newly diagnosed cases were myelodysplastic syndromes, specifically refractory anemia (RA) or refractory anemia with excess blasts (RAEB). Information on jobs or industrial activities conducted, including information on exposures to chlorinated solvents, toluene, xylene, and organic solvents. Only 4 of the cases with myelodysplastic syndrome had certain organic solvent exposure. When cytogenetic information was available and considered for those with certain and possible exposures to organic solvents, no association was found between organic solvent exposure and the occurrence of chromosomal aberrations. The investigators note that their findings were in contrast to other studies where positive associations were observed. Formal epidemiological investigations were recommended to evaluate the association between organic solvent exposure and chromosomal aberrations in patients with myelodysplastic syndromes.

In a case-control study to evaluate the role of exposure to myelotoxic agents (including exposures to organic solvents) in the development of myelodysplastic syndromes, Rigolin et al. (1998) investigated whether there was a correlation between exposures to myelotoxic agents and chromosomal changes in those diagnosed with myelodysplastic syndromes. The investigators found that “a distinct pattern of chromosome aberrations was associated with MDS arising after occupational exposures to myelotoxic agents”. Many of the chromosomal changes involved chromosomes 7, 5, and 8.

The involvement of chromosome 7 in secondary MDS has also been reported by Kuendgen et al. (2007). In their communication they note that secondary MDS “is often characterized by specific cytogenetic lesions, especially involving chromosome 7”. Chromosomal aberrations involving chromosome 7 (as well as chromosome 5) was also reported by Sandler et al. (1995) in an investigation of 158

cases of MDS patients with reported exposures to petroleum distillates with varying aromatic content. All MDS patients with chromosome 7 deletions were exposed to petroleum distillates.

In their discussion of leukemia and myelodysplastic syndromes secondary to drugs, radiation, and the environment, Levine and Bloomfield (1992) discussed the results from the largest single institution studies that had reported chromosomal aberrations in 76 to 97% of MDS/AL cases. They report that loss of all or part of chromosomes 5 and 7 are considered "the characteristic findings of secondary MDS/AL and include monosomy 5 and 7". In discussing various studies conducted to evaluate secondary MDS and acute leukemia, Levine and Bloomfield note that in a majority of investigations chromosome 7 has been observed in as many as 65% of cases, including aberrations monosomy 7, loss of the long arm, and translocations. They also report the observations of involvement of chromosome 5, the second most frequent chromosome involved. Other reported chromosomal involvement in secondary MDS and acute leukemia include chromosome 17, 21, and 11.

The various lines of evidence from these investigations suggest that the cytogenetic effects of benzene and induction of chromosomal aberrations is likely to play an important role in the development of leukemia (Hayes et al. 2001). Recent investigations continue to assess and report the frequent detection of increased chromosomal aberrations among benzene exposed leukemia patients (Paz-y-Mino et al. 2008; Kim et al. 2010; Zhang et al. 2011; Ji and Zhang 2012; Zhang et al. 2012). For additional studies and investigations on the cytogenetic effects of benzene, please see Kim et al. (2004), Holeckova et al. (2004), Zhang et al. (2002), Marcon et al. (1999), Smith et al. (1998), Zhang et al. (1996), Ciccone et al. (1993), Katz et al. (1992), Shannon et al. (1992), Cuneo et al. (1992), Robertson et al. (1991), Garson (1984), Rowley (1983) and Mitelman et al. (1981).

Exposure-Response The establishment of the putative association between a particular toxicant and a disease is dependent on the toxicology and epidemiology of the particular toxicant, along with quantitative data and information derived from industrial hygiene studies and sampling. Toxicology studies will aid in the characteristics of the disease process mechanisms and the dose-response relationship. Industrial hygiene studies and sampling provide quantitative data used in the risk and exposure assessment, including characterization of the metrics of intensity, duration, frequency, and concentrations of exposure. The epidemiologic investigations help identify increased risks for associations of exposure and disease outcomes, disease risk factors and disease confounders, as well as help characterize exposure-response relationships based on the evaluation of risks across quantitative exposure stratifications. Exposure-response establishment is not dose-response for purposes of causation.

In their international pooled analysis of the myelodysplastic syndromes and benzene exposure among petroleum workers, Schnatter et al. (2012) reported a monotonic exposure-response relationship between benzene exposure and myelodysplastic syndromes under various metrics under considerations: highest vs. lowest tertile, >2.93 vs. <or=to 0.348 part per million-years (ppm-years, Odds Ratio (OR)=4.33

(95% CI: 1.31 to 14.3); peak exposures (>3 ppm), peak vs. no peak exposure, high and medium certainty diagnosis: OR = 6.32 (95% CI: 1.32 to 30.2; and highest exposure certainty, peak vs. no peak exposure, OR = 5.74 (95% CI: 1.05 to 31.2. Interestingly, for AML (the disease most associated with benzene exposure) there was little evidence of an exposure-response relationship and there was limited evidence for an exposure-response relationship with benzene exposure and chronic lymphocytic leukemia, chronic myelogenous leukemia, or myeloproliferative disorders. In their systematic review and meta-analysis of the epidemiologic evidence on the relationship between occupational benzene exposure and leukemia risk, Khalade et al. (2010) did observe an exposure-response relationship for AML, although not statistically significant: low exposure, effect estimate: 1.94 (95% CI: 0.95 to 3.95); medium exposure, effect estimate: 2.32 (95% CI: 0.91 to 5.94); high exposure, effect estimate: 3.20 to 9.45).

Intervention Epidemiology One of the characteristics of intervention epidemiology is that the activity of intervention, whether for purposes of evaluating disease prevention or looking to reduce mortality, is applied to two or more study groups that are followed prospectively and then compared to a control groups(s) that do not receive the intervention. In the case of benzene exposed cohorts, we know from the epidemiologic literature that the lower the dose (exposure) the less disease risk.

In terms of Risk Characterization, therefore, we assemble and evaluate all of the information gathered from the descriptive epidemiology, molecular epidemiology and exposure-response processes and identify and characterize the limitations and uncertainties associated with the described risk. From an epidemiologic standpoint, we are evaluating what is both the defined magnitude and significance of the associative risk between exposure and disease and also what the health benefit would be from implementing some activity (lowering of an exposure limit, for example) to reduce the risk. As noted by Nurminen et al. (1999), intervention should "measurably show a parallel between exposure reduction and risk reduction".

Application Tables 7.2 and 7.3 apply the benzene example to the Hill criteria (Table 7.1) and associated epidemiologic strategies with the phases of the quantitative risk for the diseases Acute Myelogenous Leukemia (AML), Myelodysplastic Syndromes (MDS), and Non-Hodgkin Lymphoma (NHL). As previously discussed, the Hill Criteria are often times used to assess causality; however, they are not absolute and should be considered more as guidelines in evaluating a causal relationship. Based on these criteria, benzene has an established causal relationship with AML and probably MDS. In contrast, the evidence for an association between of benzene exposure and NHL is limited, uncertain, unstable and debatable.

Table 7.2 Applications of the Hill criteria assess association of benzene and AML, MDS, and NHL

Method		Acute Myelogenous Leukemia (AML)	Myelodysplastic Syndromes (MDS)	Non-Hodgkin's Lymphoma (NHL)
Hill criteria	Strength	Established; Sufficient; Issues: Low level exposure, metrics of exposure-cumulative, intensity, FAB ranking	Potential; Issues: MDS progressions to AML, Pre-leukemic condition; MDS are heterogeneous sets of diseases (limited data available)	Limited evidence; Debatable; Uncertain, unstable predictions
	Consistency	Most studies have had similar results; Issue of threshold, low-level exposure	Mixed; Issues: Lack of detail on MDS subtypes, inconsistent results; Low-level exposures	Results vary and results unclear; No consensus; Issues: Historical/Current Classification/ Diagnosis; Alternative risk factors; Statistical power limitations for sub-type specific analysis
	Specificity	Yes, although need to consider confounders, such as radiation	Probable/Possible; Issues: Confounders; Specificity of subtype	Limited evidence; Issues: Other identified risk factors; Confounding exposures, Classification/ Diagnosis
	Temporality	Benzene exposure occurs before development of AML; Issue of background rate	Appears that benzene exposure occurs before development of MDS	Unknown
	Biological Gradient	Has been shown; Issue of threshold, Issues: low-level exposures, metrics of exposure- cumulative, intensity	Data suggest dose-response; Issues: Low-level exposure findings, possible conflicting results with AML	Unknown
	Plausibility	Yes; Sub-chronic effects (chromosomal aberrations) increase risk	Plausible; cytogenetic effects appear strong	Unknown

Table 7.2 (continued)

Method		Acute Myelogenous Leukemia (AML)	Myelodysplastic Syndromes (MDS)	Non-Hodgkin's Lymphoma (NHL)
	Coherence	Animal studies suggestive-species variability	Mechanism is possible	Unknown
	Experimental Evidence	Animal data exists	Data not available	Data not available
	Analogy	Smoking and radiation have also been associated with development of AML; Precise mechanism of leukemogenesis not clear	Unclear. Data are limited	Unclear. Data are limited

Table 7.3 Applications of epidemiologic methods to risk assessment process regarding benzene and AML, MDS, and NHL

Method		Acute Myelogenous Leukemia (AML)	Myelodysplastic Syndromes (MDS)	Non-Hodgkin's Lymphoma (NHL)
Epidemiologic methods in risk assessment	Descriptive epidemiology	Mortality and incidence studies (cohort and case-control) reveal significant increase in risk	Limited evaluation shows potential increase in risk.	Limited evidence; Debatable; Uncertain, unstable predictions
	Molecular epidemiology	Cytogenetic studies report benzene associated with alteration of chromosomes likely condition for AML	Cytogenetic studies report benzene associated with alteration of chromosomes likely condition for MDS	Inconsistent data
	Exposure-response	Studies have reported exposure-response relationships	Studies are mixed; Issues: Findings can contrast AML at low-level exposures	Unknown; Extremely limited data
	Intervention epidemiology	No clear data	No clear data	No clear data

Summary and Conclusion

The use of epidemiology in the risk assessment process is compelling. It offers the needed human-based associative data characterizations for cohort exposures and disease association determinations. Further, epidemiology provides necessary information on cumulative, intensity, duration, and frequency metrics of exposure and the exposure-response relationship.

The current practice of relying on animal data in the risk assessment process, coupled with what Nachman et al. (2011) described as the "disconnect between available epidemiologic data and the needs of risk decision makers," compels the need for epidemiology to be part of the risk assessment process. By its nature, the discipline of epidemiology provides valuable insights into the identification, characterization and management of disease risks.

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Chapter 8
Emerging Issues in Risk Assessment

Sol Bobst

Abstract This chapter covers a brief survey of the current "Hot" topics in risk assessment. Several efforts are emerging to produce "Big Data." There are new technologies and techniques being debated and applied by the risk assessment community. Awareness of these emerging issues will help the beginner stay informed as the risk assessment community continues to evolve.

Keywords Biomonitoring · Big data · REACH · Red book · Silver book · EDSP · EPA · WoE · EBT · MoA

Student Learning Objectives:

- Learn Current Trends in Risk Assessment
- Compare and contrast different viewpoints of stakeholders
- Be prepared for future developments in the field of in Risk Assessment

Introduction

This chapter outlines some of the current dialogues that are taking place in the risk assessment community. Like any professional practice area, technology, methods, and applications change over time. With these changes, come dialogue and application of new methodologies. Changes in any professional practice are always met with a spectrum of reactions. There are those that are opposed to changes, or expect changes to happen very slowly. There are those that are more open to change with

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demonstrated application and success. Finally there are those that are eager to make change without any consultation or understanding of the consequences.

The biggest emerging issues deal with three main questions. The first question is: *What do we do with massive amounts of data*? or as commonly refer the "BIG DATA" question. The second question is: *How we can incorporate new technologies and techniques into the risk assessment process*? The third and final question is: *How do we improve decision making in the risk assessment process*? For the purposes of this chapter, the decision making process will be framed in the Weight of Evidence (WoE) versus Evidence Based Toxicology (EBT) debate. This chapter will also discuss and introduce important organizational activities that are active in the risk assessment process.

The biggest concern for regulatory stakeholders is the application of new methods to the risk assessment process. The main reason for this concern is that changes in methodologies may be too conservative, too lenient, or do not have enough information available in order to make a scientifically supported determination using the methodology.

One example of a regulatory framework by a governing body is the The European Regulation called the Registration, Evaluation, Authorization of Chemicals (REACH). Each chemical has to be registered with a dossier, that includes a risk assessment with exposure scenarios. The rule presented an enormous challenge for industry, and there were many scenarios and conditions for exposure that could exist in the supply chain environment. The result is that generic exposure scenarios were developed, based on "worse-case" conditions of exposure. The scenarios influence the risk assessment results and procedures required for safe handling and use of the chemical, and "safe use" instructions on the Safety Data Sheets (SDS). While the exposure scenarios provide a decision framework for the application of safe use and handling, the process does not address realistic exposures that actually occur in the work environment. While the end goal of safety under all conditions is seen as justified, a fair question still remains if the "worse-case" approach is over conservative, without having actual data to make decisions for protective measures.

The World of Big Data

Biomonitoring Data

Data from assays, exposures, and new experiments is now generated faster than we have the ability to understand. For instance, the fast generation of biomonitoring data in the exposure environment. Public and private institutions generate data on environmental exposures that measure chemicals, metabolites, and reaction products in human blood, milk, urine, saliva, or other tissues, across a human population (Needham 2007).

Pressure is starting to develop from Stakeholders (communities, academics, Non-Governmental Associations, and regulatory agencies) to use biomonitoring

data to make better regulatory and risk management policies and decisions. Despite the pressure to act quickly, there is debate on what methods should be used for interpreting data. There is also recognition that there need to determine how best biomonitoring (or genomic, *in vitro*) data should be utilized in a risk evaluation framework for use by regulators and industrial stakeholders.

One challenge with biomonitoring is that detection can occur at parts per billion or lower levels in biological specimens. The challenge with generating data at such low levels is to frame it in a proper context; just because data can be generated, or a chemical or biological response can be measured, does not automatically mean that the data is relevant in causing an adverse effect. This relates back to the initial building block principles in the risk assessment process discussed earlier in the risk characterization chapter. For example, the overall intended uses for biomonitoring include the provision of a metric of exposure, methods for trend monitoring, and the use as a tool to improve basic design of studies. Often media reports may share that "chemical x is detected in biological tissues." Chemical x may also be part of normal biology. Formaldehyde, for example, is formed during normal biological processes. This illustrates the point that just because a chemical can be detected in biological tissues and below risk levels, does not mean it is necessarily a concern.

Big Studies: The NHANES Study

The National Health and Nutrition Examination Survey (NHANES) is a multi-year, multi location, multi factor mega study that addresses the health of adults and children in the United States. It is coordinated by the Center for Disease Control. It uses questionnaires, datasets, and other sources of documentation to share survey results and support policy decisions. Numerous reports are released on various chemicals that maybe involved in food or environmental exposures. Interpretation of data that can have near a dozen evaluation factors is enormously challenging for risk assessors. For example, a recent survey result for the data on *Exhaled Nitric Oxide* includes the following variable factors in the table below (Table 8.1):

Table 8.1 NHANES example of variable factors (CodeBook) included in exhaled nitric oxide report

ENQ10—Breathing Problem require oxygen?
ENQ020—Problem Taking Deep Breath?
ENQ040—Smoked last hour?
ENG050—Exercised strenuously last hour?
ENQ060—Ate or drank last hour?
ENQ070– Ate NO-rich vegetables, 3 h?
ENQ080—Ate NO-rich meats, 3 h?
ENQ090—Used oral or inhaled steroids, 2 days?
ENQ100—Cough, Cold, Respiratory Illness, 7 days?
ENAATMPT—Total number of exhalation attempts

The data for exhaled Nitric Oxide has to be correlated with each of these factors. Also included are the challenges of the strength of the survey and epidemiological data collection quality, as discussed in the previous chapter. The important message is that the explosion or addition of many facets of data evaluation can provide challenges as well as quality issues for the evaluation process. There is valid concern that conclusions maybe drawn from data, without a strong Weight of Evidence (WoE) or known mechanism of action justification, as discussed in previous chapters. Omitting a robust evaluation of the strength of association, and whether or not there is a strong scientific argument for a "causation" effect, could lead to erroneous calculations and inaccurate decision making for risk assessment purposes.

New Technologies

In vitro Assay development and use for regulatory decision making is another emerging issue. Novel test methods are attractive with the advancement of technology. They may also reduce cost of studies, compared to long term animal studies. There is also pressure from community stakeholders to reduce the use of animal testing where a suitable alternative can be used. Some regulations also require the use of alternative testing methods. One example includes the use of in vitro, cell based assays for "Tier 1" screening in the Endocrine Disruptor Screening Program (EDSP) administered by the US EPA. Another example is the ban of sale of cosmetics in the EU that have been tested on animals (European Commission on Health and Consumers). The use of 'Omics' data, specifically changes in genetic or proteomic profiles based on exposures, is also a new technology that there is interest in using. Finally, computer modeling, like the EPA ToxCast™ program, or the use of QSARs for toxicological prediction are new techniques that are of interest for hazard identification and classification in risk management. The National Academy of Sciences recently published a report on "Toxicology Testing in the 21st Century" (National Academy Press 2007) with emphasis on the need to use and incorporate these new technologies for hazard identification and risk characterization.

ToxCast

To address the recognized challenge of limited capability to do animal testing for all known chemicals, as well as the interest to support alternatives to animal testing, the EPA has supported the development of computational approaches to toxicology. This includes the Toxicology Forecaster tool, or ToxCast™ The program is also focused on priorities, thus ranking testing of the most important chemicals of interest first. Their priorities are on chemicals listed by the Endocrine Disruption Screening Program (EDSP) as well as chemicals of interest listed on the inventories of the Toxic Substances Control Act (TSCA) and the Safe Drinking Water Act's

candidate contaminant list (SDWA). The EPA has conducted evaluation of over 2000 chemicals to date with over 700 high-throughput assays that cover 300 biological signaling pathways. Interpretation of such data and its complexity require a good evaluation that is time consuming.

Furthermore, ToxCast program is in early stages of evaluation. The first question ToxCast needs to answer is: Can high throughput screening and computational evaluation be an effective method for evaluating toxicity, hazard characterization, and eventually risk? To address this question, EPA initiated a "Phase I" Proof of Concept to focus on studies involving pesticides. The data can be searched publicly on EPA's Toxicity Reference Database (ToxRefDB). Once proof of concept is complete, Phase II will address the question: "How well can we use this technique across all chemicals of interest?" This will expand the testing to chemicals in industrial and consumer products, food additives, "green" products, nanomaterials, and failed drugs (that didn't reach the marketplace due to concerns during the trial phase).

ToxCast is a good example where a large body of data starts to depend on decision "systems" and frameworks that involve many stakeholders. The goal is not for one individual to make an isolated decision. Similar to the theme with the data from the NHANES study, the excitement created by new technologies and techniques, also includes the challenges of deciding how to use the data with current risk assessment decision frameworks. Also, the production of big data is forcing risk assessments to think about how the approach to risk assessment must evolve in order to address these challenges.

The Influence of Hazard Based Approaches

Many of the building blocks for risk assessment covered in this book relate to the landmark publication on risk assessment published in 1983 by the National Academy of sciences (National Academies Press 1983). This was nicked the "red book" do to its red color cover. The development of the US EPA Integrated Risk Information System (IRIS) related to the steps of the book. While the process has been followed, there have been challenges along the way. One challenge is the subjectivity of selecting data for decision-making. Another challenge that has happened repeatedly over the decades since the red book publication is the development of risk assessments based on animal data, that have mechanisms of action that are not present in humans. The most classic example is alpha 2u-globulin, which in rats can be involved in the formation of renal tumors, but does not occur in humans, which do not have this protein or mechanism of action (Dietrich 1997). The complexity of data in terms of scientific studies continued to grow.

In 2009, the national academy of sciences published the "silver book" (again named for its silver color) with the title "Science and Decisions: Advancing Risk Assessment" (National Academies Press 2009). The publication addresses topics such as the current interest in linear, non-threshold approaches in textbook

toxicology and probabilistic risk assessment, this challenges the commonly accepted idea of a threshold approach to an adverse effect. This model of thought has been presented in carcinogenesis models of toxicology. In vitro assays and other sensitive biological techniques can now measure very small levels of activity, in dose response or exposure scenarios. This relates to the questions of what is being measured and why. The importance of a threshold concept suggests that if a critical point of threshold can be determined experimentally, then a safety factor can be divided out in order to manage the exposure (risk). The argument against this approach has developed from studies in cumulative exposure, or the idea that dose and exposure can be additive, even when something is not measurable (National Academies Press 2009). One example is the formation of DNA adducts or methylated DNA that is part of a normal biological process, but at some point can become deleterious. The challenge with linear, non-threshold approaches, is that is can be used to support an argument that there can be no-safe level of exposure. The challenge lies in quantifying the risk with something that doesn't necessarily show an outright adverse effect.

Techniques in Decision Making

The Weight of Evidence (WoE) Versus Evidence Based Toxicology (EBT) Debate

Thomas Hartung (2010) and other leaders have addressed the challenge of the long lead-time (10 years) and cost to bring testing and therapy methods to markets or regulation. The value of standardization and validation is important for industrial processes, and such investment is referenced in this chapter (also important in global standardization of risk assessment). One argument to address these concerns is to have an integrated testing strategy that can weigh the balance of testing strategies. For instance, the Aspirin example, it has been fairly argued that if aspirin were to be tested for safety today under the Food and Drug Administration guidelines, it would not pass, due to the known side effects. Another challenge is the development of pharmaceuticals and managing the safety tests. Cytochrome P450s, specifically 3A4 and 2D6, are highly polymorphic, and in a large enough population size, say 1 million, there maybe some side effects found for some patients. The drug may still be beneficial for the small sub-population that the drug is targeted for. In contrast a drug not intended for a wide population use, may have trouble getting approval due to side effects in sensitive sub-populations discovered during the approval process. Those side effects may or may not be relevant for the target patients whom the drug is being developed for.

Weight of Evidence is an approach to use information supporting an understanding that maybe among several options of definitions or understanding, when uncertainty exists. Critics of Weight of Evidence often share that there is a lack of

consensus about its meaning or the subjective level of qualitative or quantitative weights that are assigned for decision making. Weight of Evidence approaches also involves validation of testing methods and strategies. In contrast Evidence Based approaches aim for creating a process that is transparent, consistent, and objective in assessing scientific evidence. Evidence based methods are widely used in medical practices. The idea is that if all data is taken together, it can be used in total to make the best decisions about risk. Criticisms of EBT approach include: lack of consensus on how integrated approaches will treat all data fairly in decision-making?; regulatory decision-making will become more intensive and difficult?; determination of how much evidence is enough evidence to conduct a systematic review?; and lack of weighted approaches may result in erroneous decision-making. The toxicology and risk assessment community continues this debate by sharing and discussing case studies of WoE versus EBT approaches.

Assessment Decision Making

Nex Gen Risk assessment (EPA) is part of the EPA's Chemical Safety for Sustainability (CSS) Research Program that will focus on fostering practical applications of new methods of risk assessment. One of the main components of the NexGen decision making process is the use of (WoE) to determine or justify how to make a risk assessment decision, based on the best supporting data available. Often times, this requires a wide search and review of literature. The challenge with this review is the debate over which studies have merit and the most value, to rely on in making decisions. There is also a lot of interest in test validation, for new technologies, in order to determine if they are reliable for making decisions. Part of the current debate on Weight of Evidence, versus Evidence Based Toxicology, is that it is not feasible to validate or due an extensive review.

The Formaldehyde Example

Recently, the EPA updated their Integrated Risk Information System file (IRIS) for the chemical formaldehyde (US EPA). Formaldehyde regulation is of interest due to classification as a carcinogen. The EPA released a draft IRIS that suggested 0.008 parts per billion as an exposure limit. This proposal was heavily criticized by both industry, and the National Academy of Sciences, due to the fact that formaldehyde is also part of normal human metabolism, at a level of approximately 1 part per billion (National Academies Press 2011). It is possible for a person to exhale formaldehyde at a level higher than what was proposed in the draft IRIS document. *Suggesting that normal human breathing and exhalation, may pose an unacceptable risk of cancer to the general population* (American Chemistry Council 2011). The formaldehyde example created a lot of public dialogue on the need for outside

peer review in the IRIS drafting process. The challenge comes from balancing a view that the EPA should be an all encompassing, competent authority to evaluate and set risk assessment levels. This view was heavily challenged with the formaldehyde example, comparing exposure to normal biological levels. The balance to this challenge is that there is also public perception that multiple stakeholders involved in peer review may have separate agendas. Some individuals believe that industry involvement in risk assessment creates conflicting or biased interest. The Draft IRIS for formaldehyde is still under review at the EPA, at the time of this publication. The benefit of this example is that dialogue continues on the role of peer review for the risk assessment process at the EPA, so that future draft IRIS proposals can be met with more validity and quality of development, by the stakeholder community.

Summary

How to use novel data, emerging technologies, and appropriate decision strategies will always be part of the risk assessment community challenges. Big Data studies provide tremendous opportunities to expand our knowledge of human health. Big Data also presents challenges in how to manage and interpret data at such a large scale. There are also dialogues and discussions on using new technologies and methodologies to improve risk assessment. The references and links provided in the text and below will help introduce the reader to these topics. Case study approaches that apply the emerging techniques will continue to be published and discussed in the toxicology and risk assessment community. The peer review of emerging issues, involving the entire risk assessment community, will determine how effective, or appropriate, these new techniques will be in application and utility.

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Chapter 9
Risk Assessment in the European Union (EU)

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Abstract Beginning in the late 1960's, EU Directives focused on the hazard identification (classification) of chemicals. In the early 1990's, the classifications of health, physical and environmental hazards were brought into the context of "risk" by Directives and Regulations that put forward the general principles, as well as a framework for the risk assessment of new and existing chemicals. In 2003, the EU *Technical Guidance Document on Risk Assessment* provided general principles of risk assessment, and, very importantly, technical details on how to perform a chemical risk assessment. The EU REACH Regulation (2006) provides the current regulatory framework for the risk assessment of chemicals manufactured or imported into the EU. Specifically, REACH sets out how importers and manufacturers are to assess and document risks that arise during the manufacture and use of chemicals, as well as how to adequately control any identified risks.

Keywords European Union (EU) · Risk assessment · REACH · ECHA · DNEL · DMEL

Student Learning Objectives

- To understand the historical aspects of human health risk assessment of chemicals in the EU
- Become familiar with EU Directives, Regulations and Technical Guidance Documents that have had significant impacts on the health risk assessment framework in the EU
- Become familiar with human health risk assessment/characterization aspects of the REACH Regulation

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The European Union—What it is

The European Union (EU) is an economic and political partnership between 28 European countries. The current EU member countries are: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom.

The EU was created following the Second World War. The intent was to foster economic cooperation based on the premise that countries that trade with one another become economically interdependent and, therefore, are more likely to avoid conflict. The resulting European Economic Community (EEC), created in 1958, consisted of Belgium, Germany, France, Italy, Luxembourg and the Netherlands. This economic union evolved into a partnership spanning policy areas, from development aid to environmental issues. In 1993 the EEC became the European Community (EC) to reflect the expansion into these diverse areas. Since 2009, this partnership has been called the EU. Since then, the EU has established common institutions: the Council (which represents national governments), the European Parliament (which represents the people), and the European Commission (an independent body that represents the collective European interest), to democratically legislate specific matters of joint interest to participating countries at a European level.

Risk Assessment of Industrial Chemicals in the EU—Historical background and Overview

The REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation (1907/2006/EC) came into force on June 1, 2007, and represents the current EU regulatory framework on risk assessment of all chemical substances manufactured or imported into the EU in quantities ≥ 1 t/year. However, before the risk assessment framework under REACH is discussed in more detail, an historical outline of previous EU Regulations[1] and Directives[2] that have had a significant impact on the development of the EU human health risk assessment process (especially those pertaining to the health hazard evaluation of chemicals—a critical aspect of the risk assessment process) is still important and will be outlined below.

[1] EU Regulations are binding and are directly applicable to all EU member states.

[2] EU Directives must be implemented by laws or regulations of the member state within a designated time period.

Council Directive 67/548/EEC (June 27, 1967)

Directive 67/548/EEC applies to substances (chemical elements and their compounds as they occur in their natural state or as produced by industry) and to preparations (mixtures or solutions composed of two or more substances) that are placed on the market in the EU. The intent of this Directive (termed the Dangerous Substances Directive; DSD) was to harmonize national measures on the classification, packaging and labeling of dangerous substances, to facilitate the establishment of a single market and to provide protection for public health and the environment. Annex I to the Directive identified (*i.e.* classified) the hazardous properties of around 8000 substances. These hazard-based classifications (known as 'harmonised' classifications) cover numerous defined physical, health and environmental endpoints. Examples of health hazard endpoints include acute oral, dermal and inhalation toxicity, dermal and respiratory sensitization, carcinogenicity, reproductive toxicity, and eye and skin irritation.

In 1979, Council Directive 79/831/EEC (the Sixth Amendment to Directive 67/548/EEC) introduced requirements for pre-marketing notification for all "new" substances to be manufactured or marketed within the Community. One of these requirements was the inclusion of health hazard classification of substances defined as "dangerous". [Note: "New" substances are those not on the European Inventory of Existing Commercial Substances (EINECS) list].

Later, in 1993, Council Directive 92/32/EEC (the Seventh Amendment to Directive 67/548/EEC) went into effect and specifically addressed risk assessment of new chemicals in that one of the Directive's objectives was "the assessment of the potential risk to man and the environment of notified substances" and that "uniform principles for risk assessment should be laid down…." However, the Directive did not provide any specifics/principles as to how the risk assessment should be performed.

Directive 1999/45/EC (May 31, 1999)

The intent of Directive 1999/45/EC was to harmonize national measures on classification, packaging and labeling of dangerous preparations…to provide protection for public health. The Directive (termed the Dangerous Preparations Directive; DPD) defines substances and preparations as dangerous to health if they are: acute lethal effects; non-lethal irreversible effects after a single exposure; severe effects after repeated or prolonged exposure; corrosive and irritant effects; sensitizing effects; carcinogenic effects; mutagenic effects; and toxic effects for reproduction. Substances are classified under the DPD with similar approach. Classification of dangerous preparations is done according to the degree and specific nature of the hazards (*i.e.* hazard-based) involved and is based on the physical-chemical, health and environmental endpoint definitions found in the Directive.

Council Regulation 793/93/EC (March 23, 1993)

Council Regulation 793/93/EEC established the requirement to assess the risk to man and the environment of existing substances. This Regulation is also known as the Existing Substances Regulation (ESR). [Note: "Existing" substances are those reported to be on the EU market between January 1, 1971 and September 18, 1981 and thus listed on European Inventory of Existing Commercial Substances (EINECS)].

Regulation 793/93/EEC introduced the following four-step process (framework) for the evaluation (*i.e.* reducing risks to man and the environment) of existing substances produced or imported in quantities of > 10 t/year: (1) Data collection; (2) Priority setting; (3) Risk assessment (evaluation); and (4) Risk reduction. Specifically, for each substance on a priority list (four such lists have been published since 1994), the "Rapporteur" (competent authorities designated by the responsible Member States) undertook the in-depth human and environmental risk assessment and, when necessary, suggested a strategy for limiting identified risks, including identifying appropriate exposure control measures. Under the ESR there are three possible risk assessment conclusions for each relevant health hazard and exposure scenario [remember: risk is a function of hazard and exposure]: (1) There is need for further information and/or testing; (2) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already; and (3) There is a need for limiting risks; risk reduction measures which are already being applied shall be taken into account. The final, independently peer-reviewed risk assessments of priority substances are referred to as EU Risk Assessment Reports (RARs).

Commission Directive 93/67/EEC (July 20, 1993) and Commission Regulation (EC) No 1488/94 (June 28, 1994)

The general principles for the risk assessment of new chemicals were presented in Commission Directive 93/67/EEC**.** The general principles for the risk assessment of existing chemicals were presented in Commission Regulation No 1488/94. The content of both the Directive 93/67/EEC and Regulation 1488/94 are essentially the same with regard to the general principles of risk assessment. Article 2 defines the four *[now]* classic steps in the risk assessment process: (1) Hazard Identification; (2) Dose—Response Assessment; (3) Exposure Assessment; and (4) Risk Characterization. Article 5 briefly outlines risk assessment for environment effects. Annex I lists the toxic (adverse) effects (*e.g.* acute and repeat dose toxicity, irritation, sensitization, carcinogenicity, and reproductive toxicity) and human populations (workers, consumers and man exposed indirectly via the environment) that shall be taken into account as part of the human health risk assessment as well as additional information relating to each of the four risk assessment steps. Lastly, Annexes II and

III provide similar information for the risk assessment for human health (based on physical-chemical properties) and the environment, respectively.

The European Commission Technical Guidance Document on Risk Assessment (2003)

The 2003 *Technical Guidance Document on Risk Assessment* (TGD), Parts I, II and III, supports legislation on the assessment of risks of chemical substances to human health and the environment. As noted earlier, only the general principles for the risk assessment of new and existing substances are outlined in Directive 93/67 and Regulation 1488/94, respectively; they do not include technical detail for conducting hazard identification, dose-response assessment, exposure assessment and risk characterization in relation to human health and the environment. The TGD was issued by the European Commission to specifically aid competent authorities to carry out human health and environmental risk assessments for new and existing substances (as well as for biocides as per Directive 98/8/EEC).

Chapter 1 of the TGD discusses general principles of risk assessment. Chapter 2 specifically deals with human health risk assessment. This chapter contains detailed information on workplace and consumer exposure assessment, chemical hazard identification (including definitions of, testing strategies for, and guidance for the evaluation of available data for health hazard endpoints such as acute toxicity, irritation/corrosion, sensitization, repeat dose toxicity, mutagenicity, carcinogenicity, and reproductive toxicity), dose-response assessment, and risk characterization for these human health hazard endpoints. Chapters 3, 4 and 6 contain detailed information on environmental risk assessment, (quantitative) structure-activity relationships (Q)SARs, and Risk Assessment Report (RAR) format, respectively. There are also seven appendices to Chapter 2 of the TGD. These describe/present, in significant detail, various topics essential to carrying-out a human health risk assessment such as occupational and consumer exposure assessment (including an overview on data and other useful information to be used for estimations of exposure and the algorithms for model estimations), toxicokinetics, and default reference values for various biological parameters for experimental animals (*e.g.* body weights, inhalation rates, food consumption, and body surface area).

Regulation (EC) No 1272/2008 (December 16, 2008)

Regulation (EC) No 1272/2008 entered into force on January 20, 2009. This Directive (referred to as the CLP) will repeal both Directive 67/548/EEC and Directive 1999/45/EC as of June 1, 2015. However, as transitional provisions, the Directive allowed for the *[hazard]* classification, labeling and packaging of substances

(in accordance with Directive 67/548/EEC) and preparations (in accordance with Directive 1999/45/EC), until December 1, 2010 and June 1, 2015, respectively. One important change in nomenclature: the CLP uses the term "mixture" instead of the term "preparation."

The CLP implements the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) that was approved by the United Nations in 2002. The GHS addresses classification of chemicals by types of hazard and proposes harmonized hazard communication elements, including labels and safety data sheets. A major aim of the GHS is that information on physical hazards and toxicity from chemicals is available in order to enhance the protection of human health and the environment during handling, transport and use of chemicals. Chapters 2, 3, and 4 of the GHS discuss, in significant detail, physical hazard endpoints (*e.g.* explosives, flammable gases, aerosols, flammable liquids, and oxidizing liquids), health hazard endpoints (*e.g.* acute toxicity, skin corrosion/irritation, respiratory and skin sensitization, carcinogenicity, reproductive toxicity, and specific target organ toxicity following repeated exposure), and environmental hazard endpoints (*e.g.* hazardous to the aquatic environment and hazardous to the ozone layer), respectively.

European Union Risk Assessment—Committees and Agencies

There are a number of independent, but often closely cooperating, scientific committees and agencies in the EU that are involved in human health and environmental risk assessment. One major function of these bodies is to provide advice on risk assessment issues (*e.g.* on chemicals, pharmaceuticals, foods, technologies, etc.) to EU decision makers to be used for preparing policy and proposals relating to consumer safety, public health and the environment. Selected EU risk assessment committees and agencies are briefly introduced below.

SCCS (Scientific Committee on Consumer Safety) provides opinions on health and safety risks (chemical, biological, mechanical and other physical risks) of non-food consumer products (*e.g.* cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products) and services (*e.g.* tattooing, artificial sun tanning).

SCHER (Scientific Committee on Health and Environmental Risks) provides opinions on health and environmental risks related to pollutants in the environmental media and other biological and physical factors or changing physical conditions which may have a negative impact on health and the environment (*e.g.* in relation to air quality, waters, waste and soils) as well as health and safety issues related to the toxicity and ecological toxicity of biocides.

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks) provides opinions on emerging or newly-identified health and environmental risks and on broad, complex or multidisciplinary issues (*e.g.* medical devices, tissue engineering, nanotechnology, blood products, methodologies for assessing

risks, etc.) requiring a comprehensive assessment of risks to consumer safety or public health and related issues not covered by other Community risk assessment bodies.

EFSA (European Food Safety Authority) provides risk assessments on food and feed safety, including nutrition, animal health and welfare, plant protection and plant health. EFSA also performs environmental risk assessments of genetically modified crops, pesticides, feed additives, and plant pests.

EMA (European Medicines Agency) has, as one of its main responsibilities, the scientific evaluation of medicines (both human and veterinary) proposed for use in the EU.

ECDC (European Centre for Disease Prevention and Control) has the responsibility to identify, assess and communicate current and emerging threats to human health posed by infectious diseases.

EEA (European Environment Agency) has as its primary objective to produce European and regional environmental data sets, environmental information and [*risk*] assessments in order to provide a sound decision basis for environmental policies in the EU.

SCOEL (Scientific Committee on Occupational Exposure Limits) has the responsibility to advise the European Commission (EC) on occupational exposure limits (OELs) for chemicals found in the workplace. Specifically, the Committee prepares toxicological/scientific evaluations of chemicals for their effects on health of workers and then gives advice on the setting of OELs based these evaluations. OELs that may be proposed (based on available, relevant health hazard data) include 8-hour time-weighted averages (TWA), short-term exposure limits (STEL) and biological limit values.

ECHA (European Chemicals Agency) has overall responsibility for the technical, scientific and administrative management of the EU REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation (EC No 1907/2006). ECHA also helps companies to comply with the legislation, advances the safe use of chemicals, provides information on chemicals and addresses chemicals of concern.

Human Health Risk assessment in the EU: The REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation (December 18, 2006)

The REACH Regulation (EC No 1907/2006) provides the current regulatory framework for the risk assessment (for both humans and the environment) for all chemicals manufactured or imported into the EU at 1 t or greater per year. The overall intent of the REACH Regulation is to ensure a high level of protection of human health and the environment.

The REACH Regulation established ECHA, amended Directive 1999/45/EC and repealed Council Regulation (EEC) No 793/93, Commission Regulation (EC)

No 1488/94 and Commission Directive 93/67/EEC (among others). The following discussion of the REACH risk of the REACH risk assessment (termed "risk characterization" under REACH) methodology will focus on human health risk characterization[3].

As in all standard risk assessment processes for chemicals, REACH human health risk assessments involve both the determination of the hazard(s) posed by the chemical as well as exposure assessment (known, estimated or modeled). The next sections will focus on the "hazard" portion of the risk equation; complete details regarding occupational and non-occupational exposure (consumer and environmental) assessment under REACH can be found in ECHA Guidance documents[4].

Human Health Risk Characterization under REACH

One of the requirements of REACH is the registration of chemical substances. If a substance (chemical) is manufactured or imported at ≥10 t per year, a chemical safety assessment (CSA) is required. As a first step in the CSA process, a hazard assessment of the substance is carried out. If, based on this hazard assessment, the substance fulfills certain hazard criteria, an exposure assessment and, ultimately, a risk characterization is required. These criteria are listed in Article 14(4) of the REACH Regulation, as amended from December 1, 2010 by Article 58(1) of Regulation (EC) No 1272/2008 (CLP Regulation). Some examples include: hazard classes 2.1–2.4; hazard classes 3.1–3.6; hazard class 4.1 and hazard class 5.1., or PBT (persistent, bioaccumulative, toxic) or vPvB (very persistent, very bioaccumulative). The results of the CSA are documented in a REACH Chemical Safety Report (CSR).

REACH Human Health Risk Characterization—Non-Carcinogens

Annex I of REACH sets out how manufacturers and importers are to assess and document that the risks arising from the substance(s) they manufacture or import are adequately controlled during manufacture and their own use(s) and that others further down the supply chain can adequately control the risks. Annex I states that one objective of the human health hazard assessment (which is one component of the chemical safety assessment; CSA) is "… to derive levels of exposure to the

[3] The reader is referred to various ECHA Guidance documents for additional information on risk characterization for the environment (*e.g.* for the aquatic and terrestrial ecosystems, predators, the atmosphere, etc.).

[4] European Chemicals Agency. (2012). Guidance on Information Requirements and Chemical Safety Assessment Chapter R.1: Environmental Exposure Estimation. Helsinki, Finland. Version 2.1.

substance above which humans should not be exposed. This level of exposure is known as the Derived No-Effect Level (DNEL)." In ECHA's REACH guidance, the DNEL has been defined as "… the level of exposure above which humans should not be exposed."

The underlying assumption for DNELs is that they represent an exposure level that is below a "no-effect" level. Therefore, DNELs are established, based on the availability of relevant data, for substances that exhibit a threshold dose-response relationship. In a threshold dose-response relationship, there are considered to be low doses/concentrations of a substance that do not produce an observable/measureable response (*i.e.* effect) in the exposed population. A response is not observed until the dose/concentration reaches a "threshold"; at that threshold dose/concentration, and greater, one can detect an observable/measureable response(s). For example, most non-carcinogens are assumed to follow a threshold dose response relationship. On the other hand, DNELs are not derived for substances that exhibit a non-threshold dose-response relationship (*i.e.* substances described as having a "non-threshold mode of action"). Examples of these types of substances, as per REACH, include mutagens and carcinogens (see: discussion of DMELs).

For the derivation of DNELs, all available health hazard data for the substance needs to be reviewed and critically evaluated. Health hazard data that support the derivation of DNELs may come from a variety of sources: Human studies (epidemiological studies, case reports, clinical studies, etc.), experimental animal studies (from well-conducted acute, subacute, subchronic and chronic general toxicity studies and from "specialized" toxicity testing such as for dermal sensitization, neurotoxicity, and reproductive and developmental toxicity, etc.), *in vitro* studies, and non-testing sources such as quantitative structure-activity relationships (QSAR), and read-across (*e.g.* analog and category approaches). The critical evaluation of this health hazard information should focus on the identification of each study's No-Observed Adverse Effect Level (NOAEL), or other relevant dose descriptor (see below), to be used as the basis (sometimes referred to as the point of departure; POD) for DNEL derivation.

Based on the available, relevant health hazard data, DNELs are derived for one (and often more) human exposure pattern(s) previously determined to be associated with an exposure scenario (ES) for the substance. As per ECHA guidance, human exposure patterns consist of four elements:

Exposed Population: Workers and/or the general population (includes consumers, persons liable to exposure via the environment, and certain vulnerable sub-populations such as children and pregnant women).

Route of Exposure: Inhalation, dermal, and oral (ingestion).

Duration of Exposure: Acute (a single exposure or exposure lasting from minutes to a few hours) and long-term (repeated, and in some cases, continuous exposure over months to years).

Effect: Local (effects observed at the site of first contact, even if the substance is systemically available) and systemic (effects observed at a site(s) distant from the site of first contact—*i.e.* the substance is absorbed and becomes systemically available).

The various combinations of these four elements can lead to a number of potential human exposure patterns such as (examples): Worker/Inhalation/Long-term/Systemic health effect; General population (consumer)/Dermal/Long-term/Systemic health effect; and Worker/Dermal/Acute/Local health effect. A DNEL will need to be derived for each of these exposure patterns that is determined relevant for the chemical being registered (based on the availability of relevant health hazard data).

Current ECHA Guidance outlines a 4-step process for deriving DNELs[5]. These steps are: (1) Gather typical dose descriptors from the available and relevant studies (*i.e*. NOAEL, NOAEC, etc. from human or experimental animal studies) on the different human health endpoints; (2) Decide on the mode of action (threshold or non-threshold); (3) Derive DNELs for all the threshold health endpoints; and (4) Identification of the leading health effect and the corresponding DNEL (*i.e.* this DNEL is used in the chemical risk characterization process of the CSA).

Step (3), above, has two parts. In part 1, Modification ("correction"), when necessary, of the selected dose descriptors for differences in bioavailability between experimental animals and humans, if route-to-route extrapolation is needed, for differences in exposure durations between experimental animals and humans, and differences in respiratory rates between workers at rest vs. during light activity (only relevant for derivation of inhalation DNELs). In part 2, the now "corrected" dose descriptors from part 1 are modified, as necessary, by the application of assessment factors (AFs) to obtain the health endpoint-specific DNEL(s). AFs are numerical values used to address the differences (uncertainties) in the extrapolation of experimental *[animal or human]* data to the relevant human exposure situation (*i.e.* the identified human exposure pattern(s)). Under ideal circumstances, these differences are addressed using substance-specific AFs derived from health hazard and/or toxicokinetic information on the substance. However, in the vast majority of cases, the data needed to derive these substance-specific AFs are not available so, default AFs are most often used in this step [note: default AFs differ for systemic and local health effects—see ECHA, 2012 for specific details]. ECHA Guidance (Chapter R.8) has identified the following five areas of differences/uncertainties that need to be addressed as part of the DNEL derivation process: interspecies differences, intraspecies differences; duration of exposure differences (*e.g.* subchronic to chronic, etc.), need to extrapolate from a LOAEL to NOAEL (because the NOAEL is the preferred starting point for DNEL derivation), and quality of the whole database (based on the available dataset for the substance).

The overall AF used in the derivation of DNELs is the product of the AFs for each of these five areas: Overall $AF = AF_1 \times AF_2 \times AF_3 \times AF_4 \times AF_5$. The corrected dose descriptor and the overall AF are used in the following way to derive each endpoint-specific DNEL:

$$\text{DNEL} = \frac{\text{"Corrected" Dose Descriptor}}{\text{Overall AF}}$$

[5] For complete details on ECHA guidance on DNEL derivation, see: European Chemicals Agency Guidance on Information Requirements and Chemicals Safety Assessment. Chapter R.8: Characterisation of Dose [Concentration]-Response for Human Health. (Version 2.1; 2012). Helsinki, Finland.

Following the derivation of all endpoint-specific DNELs, the leading health effect and the corresponding critical DNEL for that effect should be selected for each relevant human exposure pattern(s). The critical DNELs are generally the lowest DNEL derived for each relevant human exposure pattern.

DNELs are used in the quantitative risk characterization step of the REACH CSA. The known or estimated exposure of each human population known to be, or likely to be, exposed to the REACH-regulated chemical(s), as indicated by the relevant human exposure patterns, is compared to the appropriate DNEL. This comparison is termed the Risk Characterization Ratio (RCR):

$$\mathrm{RCR} = \frac{\mathrm{Exposure}}{\mathrm{DNEL}}$$

Risk to the human population under consideration (i.e. for that human exposure pattern) can be shown to be controlled if the exposure is less than the DNEL (*i.e.*RCR is <1). If exposure is greater than the DNEL (*i.e.* RCR >1), the risk is not controlled. In cases where the RCR is >1, REACH allows for the implementation of risk management measures (RMMs) and/or operational conditions of use (OCs). The main effect of the implementation RMMs and/or OCs is to prevent, control, or reduce exposure of humans and/or the environment which, in turn, significantly reduces or eliminates *[health]* risk(s) posed by the substance.

RMMs include any action that is introduced during manufacture or use of a substance in order to prevent, control, or reduce exposure of humans and/or the environment. RMMs, and their proper implementation, are of critical importance for the safe use of substances by workers and consumers. RMMs fall into three different categories: engineering controls, administrative controls and personal protection equipment (PPE). OCs include any action that prevails during manufacture or use of a substance that as a side effect might have an impact on exposure of humans and/or the environment. Examples of OCs, applicable to both worker and consumer use of substances, include the amount of substances used/applied, the duration and/or frequency of use of a substance during a particular workplace or consumer process/task, the concentration of the substance in a mixture, the temperature at which the task/process is carried-out (*i.e.* may impact volatilization of the substance), containment of a process (*i.e.* closed process in the workplace), and the specification of the surroundings where the substance is used (*i.e.* indoor or outdoor use). If RMMs and/or OCs are implemented in the risk characterization step, they must be transmitted, and used, by downstream users of the chemical.

REACH Human Health Risk Characterization—Carcinogens and Mutagens

In addition to the discussion of DNELs, REACH Annex I (Sect. 1.4.2) states that: "If it is not possible to identify [*i.e. derive*] a DNEL, then this shall be clearly stated and fully justified." DNELs may not be able to be derived for substances for

a number of valid reasons such as lack of available/appropriate health hazard data for the substance, or that the substance is a mutagen or carcinogen that acts via a "non-threshold mode of action." Regarding the latter instance, REACH Annex I states in section 6.5 that "For those human effects … for which it was not possible to determine a DNEL…, a qualitative assessment of the likelihood that effects are avoided when implementing the exposure scenario shall be carried out." Under the qualitative [*human health*] assessment approach for non-threshold mutagens and carcinogens, a semi-quantitative approach may be included whereby a DMEL (Derived Minimal Effect Level) is developed, assuming that there are data allowing it.

A DMEL is a reference risk level which is considered to be of very low concern. Exposure levels below a DMEL are, therefore, judged to be of very low concern. Although there is no EU legislation setting the 'tolerable' risk level for carcinogens, cancer risk levels have been set and used in different contexts (both applied within and outside the EU). Based on these observations, ECHA Guidance states that cancer risk levels of 10^{-5} and 10^{-6} could be seen as indicative tolerable risks levels when setting DMELs for workers and the general population, respectively.

DMELs, as with DNELs, are derived as part of the human health hazard assessment process of the CSA. For the derivation of DMELs, all available health hazard data for the substance needs to be reviewed and critically evaluated. As per ECHA Guidance, health hazard data that support the derivation of DMELs may come from various sources including: Human studies (*e.g.* epidemiological studies), experimental animal studies, read-across (analog or category approaches), and the use of the principle of the threshold of toxicological concern (TTC). The TTC approach is used in human health risk assessment to set threshold exposure values for chemicals (based on their structure and the known toxicity of chemicals that share similar structural characteristics) below which there is a very low probability of adverse effects. Based on the available, relevant health hazard data, DMELs are derived for human exposure pattern(s) previously determined to be associated with an exposure scenario (ES) for the mutagenic/carcinogenic substance. Again, human exposure patterns consist of the four elements mention previously in details: (a) exposed population; (b) route of exposure; (c) duration of exposure; and (d) effect.

The various combinations of these four elements can lead to a number of potential human exposure patterns such as (examples): Worker/Inhalation/Long-term/Systemic health effect; General population (consumer)/Dermal/Long-term/Systemic health effect; and Worker/Dermal/Acute/Local health effect. For each of these exposure patterns that is determined to be relevant for the chemical being registered, a DMEL will need to be derived (based on the availability of relevant health hazard data).

Current ECHA Guidance outlines a 4-step process for deriving DMELs from results of studies in experimental animals[6]. These steps are: (1) Gather typical dose

[6] For complete details on ECHA guidance on DMEL derivation, see: European Chemicals Agency Guidance on Information Requirements and Chemicals Safety Assessment. Chapter R.8: Characterisation of Dose [Concentration]-Response for Human Health. (Version 2.1; 2012). Helsinki, Finland.

descriptors from the available and relevant studies (*e.g.* examples of common toxicological values used as dose descriptor starting points for the derivation of DMELs include: Relative Risk (RR) and Odds Ratio (OR) from human epidemiological studies and the BMD/ $BMDL_{10}$ or $BMCL_{10}$ from experimental animal studies); (2) Decide on the mode of action (DMELs are derived for substances that exert their effects via a non-threshold MOA); (3) Derive DMELs for the non-threshold health endpoints [this step have two semi-quantitative approaches for DMEL derivation outlined in ECHA Guidance: The "Linearised" approach and the "Large Assessment Factor" ("EFSA") approach]; and (4) Select the leading health effect and the corresponding DN(M)EL (used in the chemical risk characterization process of the CSA).

The "Linearised" approach gives a DMEL(s) that represents an exposure level where the likelihood that effects, as assessed by excess lifetime cancer risk, are avoided and are, thus, considered to be of "very low concern." The T25 (*i.e.* the chronic daily dose in mg per kg bodyweight which will give 25% of the animals tumors at a specific tissue site, after correction for spontaneous incidence, within the standard life span of that species) is the preferred default starting point; however, the BMD_{10} (*i.e.* the benchmark dose associated with a 10% response adjusted for background) can also be used under certain circumstances. In some cases, the identified dose descriptors may need to be modified to a "corrected" dose descriptor based on: (1) Differences in bioavailability between experimental animals and humans; (2) Route-to-route extrapolation, if needed; and (3) Differences in respiratory rates between workers at rest vs. during light activity.

The now "corrected" dose descriptors from part 1 are modified, as necessary, by the application of assessment factors (AFs) to obtain the DMEL(s). AFs are numerical values used to address the differences (uncertainties) in the extrapolation of experimental [*animal or human*] data to the relevant human exposure situation (*i.e.* the identified human exposure pattern(s)). ECHA Guidance identifies the following four areas of differences/uncertainties that need to be addressed as part of the DMEL derivation process via the "Linearised" approach: interspecies differences; intraspecies differences; duration of exposure differences; and quality of the whole database. As per ECHA Guidance, default AFs for intraspecies differences, duration of exposure differences, and quality of the whole database are set equal to 1; interspecies differences are based on the principle of "allometric scaling" which is described in detail in ECHA Chapter R.8.

The last step in the derivation of a DMEL (*e.g.* for non-threshold carcinogens) using the "Linearised" approach is to apply a "high to low dose" risk extrapolation factor (HtLF) to the corrected dose descriptor(s) to obtain DMEL(s) for the relevant human exposure pattern(s). For workers at an excess lifetime cancer risk $= 10^{-5}$, the HtLF = 25,000 when the starting dose descriptor is T25 and 10,000 when the starting dose descriptor is the BMD_{10}. For the general population (*e.g.* consumers) at an excess lifetime cancer risk $= 10^{-6}$, the HtLF = 250,000 when the starting dose descriptor is T25 and 100,000 when the starting dose descriptor is BMD_{10}. As an example, for a worker at 10^{-5} excess lifetime cancer risk starting with T25, the DMEL calculation would look like:

$$\text{DMEL} = \frac{\text{“Corrected” T25}}{\text{Overall AF} \times 25{,}000}$$

The “Large Assessment Factor” (“EFSA”) approach results in DMELs representing exposure levels where the likelihood that carcinogenic effects are avoided is appropriately high and, thus, of low concern from a public health point of view. The $BMDL_{10}$ (*i.e.* the corresponding lower limit of a one-sided 95% confidence interval on the benchmark dose) is the preferred starting point. As per ECHA Guidance, the same dose descriptor modifications are to be applied, as necessary, as in the “Linearised” approach. Following any modification of the dose descriptor, AFs are applied to account for uncertainties/differences with respect to: interspecies differences; intraspecies differences; nature of the carcinogenic process (*i.e.* inter-individual human variability in cell cycle control and DNA repair); and “point of comparison” (*i.e.* the T25 and $BMDL_{10}$ are not considered to be NOAELs). ECHA Guidance suggests a default value of 10 for each of these differences/uncertainties. As an example, the DMEL derivation for the general population (including consumers) using this approach (with defaults) would look like:

$$\text{DMEL} = \frac{\text{“Corrected” BMDL10}}{10 \times 10 \times 10 \times 10}$$

DMELs (derived either by the “Lineraised Approach” or the “Large Assessment Factor”) approach are used in the semi-quantitative risk characterization step of the REACH CSA. The exposure of each human population known to be, or likely to be, exposed to the REACH-regulated chemical(s) is compared to the appropriate DMEL. This comparison is also termed the risk characterization ratio (RCR):

$$\text{RCR} = \frac{\text{Exposure}}{\text{DMEL}}$$

If exposure is $<$DMEL, exposure is said to be controlled to a risk level of low concern. If exposure is $>$DMEL, risk is not controlled. Again, in cases where the RCR is >1, REACH allows for the implementation of risk management measures (RMMs) and/or operational conditions of use (OCs). The main effect of the implementation RMMs and/or OCs is to prevent, control, or reduce exposure of humans and/or the environment which, in turn, significantly reduces or eliminates [*health*] risk(s) posed by the substance.

Summary

The EU has had a relatively long history of issuing Directives and Regulations pertaining to the health, physical-chemical and environmental hazard assessment (evaluation) of chemicals and preparations (mixtures). One major objective of these

early Directives/Regulations was to harmonize the [*hazard*] classification, packaging and labeling of substances and preparations (mixtures) to provide protection for the public's health. A logical extension of these hazard-based endeavors was the development of a framework, and eventually specific guidance/methodology, for the health and environmental risk assessment of these substances and preparations (mixtures). Out of this came the REACH Regulation which now provides the current EU regulatory framework for the risk assessment (for both humans and the environment) of all chemicals manufactured or imported into the EU at 1 t or greater per year.

In addition to providing the current EU regulatory framework for chemical risk assessment, the REACH Regulation has also brought focused attention on many specific aspects important to human health risk assessment including: (1) The development and use of exposure models for the assessment of occupational and consumer exposures to chemicals; (2) The use of alternative approaches for obtaining health hazard data including testing using *in vitro* methodologies and by using chemical Quantitative Structure Activity Relationship (QSAR) and chemical "grouping" approaches based on chemical structures; and (3) The increased awareness of the need to use the best available data in risk assessments by critically assessing/evaluating the quality, methodology, results reporting and conclusions of available health hazard studies (for example, by using the Klimisch *et al.,* scoring system as described in: *Regulatory Toxicology and Pharmacology*, 25:1–5, 1997). Lastly, the derivation and dissemination of DNELs for numerous chemicals under REACH, especially for those chemicals not already having health-based occupational exposure limits (OELs), has recently led to much debate in the scientific community regarding whether or not worker-inhalation DNELs should be used as, or even replace, existing health-based OELs established by global consensus and regulatory bodies.

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Chapter 10
Brief Survey of Global Approaches for Risk Assessment

Silvia Berlanga de Moraes Barros and Sol Bobst

Abstract The purpose of this chapter is to create awareness of global regulatory approaches outside the United States and the EU. This will make the reader aware of regulatory approaches and Risk Assessment in other regions. Risk assessment is an emerging concept in many regions of the globe. Most countries still legislate based in chemical hazard than risk. The initiatives and regulatory approaches in these countries are explored based on the knowledge and detail available. The chapter covers these topics in a survey style approach. The reader is encouraged to visit the references for more detailed information.

Keywords CAN · CEPA IPCS · Latin America K-REACH MERCOSUR Regulatory · Risk Assessment · SAICM WHO

Student Learning Objectives

- Obtain an understanding of global regulatory frameworks
- Learn about regulatory agencies in other countries

Introduction

It is important for the beginner in Risk Assessment to be aware of all the global regulatory approaches, North America and Europe have the most mature regulatory frameworks. However, other regulatory guidelines are emerging globally that the beginner should be aware of. This global awareness will help future regulators and risk assessors create global standards and approaches to risk assessment. Standard-

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ization in regulatory approaches supports efficient governance as well as reduces regulatory burdens for international trade and regulatory purposes. While this list is not exhaustive of every country, it does provide a strong basis for individuals to learn the scope of regulatory frameworks in a global context. At a minimum, it is also a useful listing resource.

Global Guidance for Regulatory Risk Assessment

WHO-IPCS

On an international level of cooperation, the World Health Organization (WHO) has an International Programme on Chemical Safety (IPCS)[1]. For developing nations without a risk assessment program, the IPCS is an organization that will help establish standards in that country. There are several focus areas of the World Health Organization. One includes the Health Impacts of Chemicals, with a focus on chemicals of major public health concern. They also provide resources on assessment and classification.

The United Nations

The United Nations also has a working committee on the Strategic Approach to International Chemicals Management (SAICM)[2]. The goal is similar to the World Health IPCS programme, to develop international guidance and standards on the management of chemicals.

Latin America

The risk assessment process is not well developed in Latin America Countries. Although the risk of exposure to different chemicals categories is mentioned in the legislation of most of the countries there is no directive that clearly indicate how to proceed with the risk assessment process.

Latin America (LA) comprises the countries of the South and Central America, Mexico in the North America continent and many of the Caribbean countries. Although many legislations exist in these countries indicating the need for risk evaluation with respect to exposure to chemicals in the various exposure scenarios there is no specific indication on how to implement the risk assessment process. Many agreements exist among LA countries with some indicating regulatory rules for food, cosmetics and pharmaceutical products trading. However, differently from the European Union, there is no regulatory action that clearly indicates the proce-

[1] http://www.who.int/ipcs/en

[2] http://www.saicm.org

dures to be followed by all countries regarding risk assessment process. There is an increasing effort in LA countries to implement the Global Harmonization System (GHS) as part of the risk assessment process. The following paragraphs describe the main trading blocs organization in South America and some of the actions regarding the implementation of the Global Harmonization System in the countries.

Latin America Regional Trading Blocs

Mercosur

Latin America region encompasses 20 independent coutries, namely Argentina, Bolívia, Brasil, Chile, Colombia, Costa Rica, Cuba, Equador, El Salvador, Guatemala, Haiti, Honduras, México, Nicarágua, Panamá, Paraguai, Peru, Venezuela, Uruguay and Dominican Republic. In 1991, by the Treaty of Asunción, an economic and political agreement among Argentina, Brazil, Paraguay and Uruguay was created (the Southern Common Market—MERCOSUR) and amended by the Treaty of Ouro Preto in 1994. In 2012, Venezuela was incorporated in the Mercosur and Bolivia signed a protocol of adhesion. MERCOSUR comprises also Chile, Colombia, Peru, Ecuador, Guiana and Suriname as associated States.

According to Article 1 of the Treaty of Asunción, MERCOSUR treaty implies:

1. The free movement of goods, services and productive factors between countries through, inter alia, the elimination of customs duties and of non-fare restrictions to the movement of goods, and any other measure having equivalent effect;
2. The establishment of a common external fare and the adoption of a common commercial policy towards third States or groups of States and the coordination of positions in regional and international economic and trade forums;
3. Coordination of macroeconomic and sectorial policies between States Parties—foreign trade, agricultural, industrial, fiscal, monetary, foreign exchange and capital, services, customs, transport and communications and others that agree—in order to ensure adequate conditions of competition among States Parties;
4. The commitment of States Parties to harmonize their legislation in the relevant areas in order to strengthen the integration process.

Among others the Mercosur treaty has the objective of harmonization of legislations[3].

Andean Community (CAN)

The Andean Community (Comunidad Andina—CAN) is another partnership organized in Latin America that entails the participation of Bolivia, Colombia, Ecuador and Peru. CAN is a community of countries that joined together voluntarily for the purpose of achieving integral, more balanced and autonomous development through Andean, South American and Latin American integration. One of the objectives of CAN is to promote the balanced and harmonious development of the member countries under equitable conditions through integration and economic and social cooperation[4].

[3] http://www.mercosur.int/t_ligaenmarco.jsp?contentid=4823&site=1&channel=secretaria.

[4] http://www.comunidadandina.org/.

Chemical Risk Assessment implementation in Latin America

Implementation of the Global Harmonized System in Latin America Countries- The federal government of Brazil instituted in 2007 an inter-ministerial task force coordinated by the Ministry of Industrial Development and external Commerce to implement the Global Harmonizes System in Brazil. The first version of the technical document that establish the criteria for the Classification System of Dangerous Products was published in September 2009 by the Brazilian Association of Technical Regulation (Technical regulation 14725–2 ABNT—Associação Brasileira de Normas Técnicas). This rule applies to all pure chemicals and mixtures and aims to provide information about the safety of chemicals to human and environmental health, to indicate the procedures for labeling of chemical products and also how to organize the safety data sheets[5].

Isolated actions were taken in some LA countries to introduce the GHS and risk assessment concept in the work place legislation.

Uruguay incorporated the risk assessment concept to the Act 307/009 (modified by Act 346/2011) indicating the minimal requirements for the worker health protection and safety considering occupational chemical exposure risk. The Act indicates that both hazard and exposure must be taken in account in the risk assessment process. Later on in 2011 this regulation was amended indicating that all companies included in the original decree will have six months to develop a plan for GHS implementation[6, 7].

In Brazil the Administrative rule 229 of 2011 of the Ministry of Labor and Employment (SIT 229/2011) indicates that chemicals in use at the workplace should be classified for hazards to safety and health of workers in accordance with the criteria established by the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations. ABNT NBR 14725 is part of the effort to implement the Globally Harmonized System (GHS) of information of hazardous chemicals[8].

Mexico published in 2011 the NMX-R-019 (NMX-R-019-SCFI-2011) that sets out the criteria for classifying chemicals according to their physical, health, and environmental hazards[9]. It also provides the elements of a uniform hazard communication system for chemical products, labeling requirements, and safety data sheets. In accordance with the third edition of the Global Harmonized System of United Nations this rule do not apply to pharmaceutical products, food additives, cosmetics, pesticide residues in food and danger residues[10]. The application of this rule by the industry is not mandatory, meaning GHS can be used on a voluntary basis and is not enforced. In Mexico chemical substances and products are legislated by six different

[5] http:www2.iq.usp.br/pos-graduacao/images/documentos/seg_2_2013/nbr147252.pdf.

[6] http://archivo.presidencia.gub.uy/_web/decretos/2009/07/T1397%20.pdf.

[7] http://archivo.presidencia.gub.uy/sci/decretos/2011/09/mtss_225.pdf.

[8] http://www.normaslegais.com.br/legislacao/portariasit229_2011.htm.

[9] http://trabajoseguro.stps.gob.mx/trabajoseguro/boletines%20anteriores/2011/bol039/vinculos/NMX-R-019-SCFI-2011.pdf.

[10] http://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev03/Spanish/00-intro-sp.pdf.

regulatory agencies making the legal regime governing chemical substances a very much complicated and often confusing subject matter, due to the number of laws and agencies that regulate chemical substances, an Inter-Secretarial Commission for the Control of the Processing and Use of Pesticides, Fertilizers and Toxic Substances (Comisión Intersecretarial para el Control del Proceso y Uso de Plaguicidas, Fertilizantes y Sustancias Tóxicas (CICLOPLAFEST)) was created in 1987[11].

Chemical substances and products are governed by a number of overlapping laws and regulations and fall under the jurisdiction of six different regulatory agencies: (1) the Health Secretariat (Secretaría de Salud (*SSA*)); (2) the Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (*SAGARPA*)); (3) the Secretariat of the Environment and Natural Resources (Secretaría del Medio Ambiente y Recursos Naturales (*SEMARNAT*)); (4) the Secretariat of the Economy (Secretaría de Economía (*SE*)); and to a lesser extent (5) the Secretariat of Communications and Transport (Secretaría de Comunicaciones y Transportes (*SCT*)) and (6) the Secretariat of Labor and Social Welfare (Secretaría de Trabajo y Previsión Social (*STPS*)). As a consequence, the legal regime governing chemical substances remains one of the most complicated and often confusing subject matters of Mexican environmental law[11] The CAN regulated the production of procedures, trade of cosmetics and hygiene products among the countries considering the possible risk of these products to human health, without mentioning how these risks were to be evaluated (Rules 516 and 706). No mention is made in these rules regarding GHS application[12]. CAN have developed draft regulations based on the 13th revised edition of the UN Model regulations, the European Agreement concerning the International Carriage of Dangerous Goods road and the Regulations concerning the International Transport of Dangerous Goods by rail that still under consideration.

Colombia implemented the GHS for transport of dangerous goods as informed in the UNECE website[13]. In Equator the rule NTE INEN 2266 incorporates the GHS for the production, marketing, transportation, storage and handling of hazardous materials in mandatory mode[14]. Argentinean rule IRAM 41400 of the Argentine Institute of Standardization and Certification uses the GHS concept in the Material safety data sheet for chemical substances.

The resolution 41/09 of the Common Market Group (GMC) of the Mercosur signed in 2009 a covenant with the European Community (ECONORMAS MERCOSUL) to develop, among others objectives, a project to implement the GHS in the countries that takes part of this common market. Within this objective two activities are ongoing, namely, (i) to promote the adoption of the international guidelines of the GHS and (ii) to strengthen and create local capacity for the analysis of chemical substances and strengthen the infrastructure of available laboratories for the implementation of the system[15].

[11] http://www.cec.org/lawdatabase/mx11.cfm?varlan=english#4.

[12] http://www.comunidadandina.org/Seccion.aspx?id=145&tipo=TE&title=productos-cosmeticos.

[13] http://www.unece.org/trans/danger/publi/ghs/implementation_e.html#c25760.

[14] http://law.resource.org/pub/ec/ibr/ec.nte.2266.2010.pdf.

[15] http://www.sice.oas.org/trade/mrcsrs/resolutions/Res4109_p.pdf.

Another project under the financial support of the Interamerican Bank of Development (BID) is under implementation to develop a regional strategy for the handle and market of chemical products in the region. With participation of Mercosur countries and Chile the purpose of the project is to develop and adopt a regional strategy for GHS implementation and compliance with the requirements of REACH. The project goal is to contribute to the promotion of intraregional trade and exports to third countries chemicals and increasing their competitiveness with a focus on sustainable development and people safety[16].

In Brazil the chemical classification of pesticides is still made by hazard criteria and although not specified follows the guidelines of OECD. A new legislation is under preparation by the Brazilian Health Surveillance Agency that introduces the concept of risk assessment in the registration process for pesticides.

The resolution 326, December 9, 2005, from the Brazilian Health Surveillance Agency (ANVISA) indicates the need for risk assessment of house use pesticides. This resolution only provides a general mention that risk assessment should be performed with these products. But no guideline on how to proceed on the different steps of the risk assessment process is detailed[17]. In 2012 ANVISA published the guideline for the Safety Evaluation of Cosmetics Products[18] including the concept of risk assessment for the safety evaluation of cosmetics as described by Rogiers; Pauwels, 2008.

A guideline for nonclinical studies to be conducted for the toxicological and pharmacological safety evaluation of pharmaceuticals was published in 2013 by the Brazilian Health Surveillance Agency with the aim to harmonize the Brazilian legislation with other regulatory agencies like the Food and Drug Administration and the European Medicines Agency and also International organizations like the International Conference on Harmonization, the Organization for Economic Cooperation and Development, the National Cancer Institute and the World Health Organization[19].

Latin America Section Summary

Many initiatives in LA countries have been put in motion to implement the application of both GHS and REACH systems in the legislation mainly in the prevention of occupational health diseases and also the regional and international trading of chemical substances. Besides that the concept of risk is only mention in a general

[16] http://www.iadb.org/es/proyectos/project-information-page,1303.html?id=rg-t1687.

[17] http://www.aladi.org/nsfaladi/normasTecnicas.nsf/09267198f1324b64032574960062343c/965452cef2650e5c032579e40049597b/$FILE/Port%20326.pdf.

[18] http://portal.anvisa.gov.br/wps/wcm/connect/04707f804e1c33cea541b7c09d49251b/Guia_cosmeticos_grafica_final.pdf?MOD=AJPERES).

[19] http://portal.anvisa.gov.br/wps/wcm/connect/e0f1d9004e6248049d5fddd762e8a5ec/Guia+de+Estudos+N%C3%A3o+Cl%C3%ADnicos+-+vers%C3%A3o+2.pdf?MOD=AJPERES.

manner in the legislations and baring a few exceptions is not applied in LA countries as a scientific method to implement human and environmental health.

Canada

Health Canada and Environment Canada

The Government of Canada established a Decision Making Framework for Identifying, Assessing, and Managing Health Risks in August of 2000. Environment Canada, manages toxic substances under the Canadian Environmental Protection Act, or CEPA 1999. Risk Management is regulated under the Assessment of Substances section of CEPA 1999. The Domestic Substance List (DSL) maintains the inventories of substances that are considered to pose a threat to human or environmental health. The Canadian regulatory frameworks are well developed, with science based approaches. The reader can find more information at the hyperlinked websites. Health Canada Decision Making Framework: http://www.hc-sc.gc.ca/ahc-asc/pubs/hpfb-dgpsa/risk-risques_tc-tm-eng.php. Environment Canada Assessment of Substances CEPA 1999 http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&n=EE479482–1&wsdoc=16C8586D-F376-5225-C45C-6EAC80B5E0B9.

Asia Pacific

Australia

For industrial chemicals, the August Government manages chemical risk assessment through the Department of the Environment, as well as the Department of Health and Ageing. This is though through the National Industrial Chemicals Notification and Assessment Scheme (NICNAS), and the Australian Pesticides and Veterinary Medicines Authority (APVMA). Currently, the regulations are being updated by the Council of Australian Governments (COAG) Chemical Reforms.

New Zealand

Risk Assessment Frameworks are published and available under the Department of Food Safety and the New Zealand Environmental Protection Authority. The reader is encouraged to visit the site for more information. The New Zealand Environmental Protection Agency has its own historical classification system, including the Chemical Classification and Information Database (CCID) that maintain classifications according to Hazardous Substances and New Organisms (HSNO) regulations. Chemicals allowed in New Zealand are managed on the New Zealand Inventory of Chemicals (NZIoC).

India

India has numerous chemical legislations, and has also received global attention on chemical risk management due to the Bhopal Gas incident in 1984. Laws regulating chemicals include the Environment Act of 1986; Hazardous Chemical Rules Act of 2000; and Chemical Accidents Amendment of 1996. Currently, none of the governing ministries have managed databases or inventories.

Indonesia

In Indonesia, chemical regulations are managed by the Ministry of Environment; their focus appears to be on hazard management. The website for the department is http://www.menlh.go.id/

China

There are several inventories of chemicals regulated in China, a searchable database is managed at this website: http://cciss.cirs-group.com/

In 2011 The Chinese Government published Decree 591 "Regulations on Safe Management of Hazardous Chemicals in China. It is a complex piece of legislation that includes multiple governing bodies. The legislation covers the Hazard Communication (GHS) requirement, New Chemicals and Dangerous Goods, Food Safety, Cosmetics, Occupational Health, Plastics and Plasticizers, and Coatings. Chemicals will have to be registered in a "China REACH" style of legislation. Legislative Authority experts are centered at the Chemical Registration Center (CRC) of the Ministry of Environmental Protection (MEP) and the State Administration of Work Safety (SAWS) of the National Registration Center for Chemicals. An English translation of the entire regulation is available at this website: http://www.cirs-reach.com/China_Chemical_Regulation/Regulations_on_Safe_Management_of_Hazardous_Chemicals_2011_English_Translation.html.

Philippines

The Phillippines has an Inventory of Chemicals and Chemical Substances (PICCS), which is administered by the Environmental Management Bureau.

Vietnam

Vietnam has been developing and updating chemical regulation laws over the past few years. In 2011, Decree No. 26/2011/ND-CP was passed that includes inventory lists and chemicals that are limited in production and trade conditions. The lists also

include chemicals subject to declaration and toxic chemicals which require control slips for purchase. Decree No. 108/2008/ND-CP contains a list of banned chemicals. The government website for chemical management is located here: http://cuchoachat.gov.vn/Trangchủ.aspx

Japan

In Japan, chemical management and risk assessment in Japan is managed by the National Institute of Technology and Evaluation, administered under the title of Chemical Risk Information Platform, it can be accessed at this site: http://www.safe.nite.go.jp/english/db.html.

Korea

The Korean Ministry of Environment has established a registration, evaluation program of chemical substances very similar to the European REACH regulations. It is so similar, that it is called K-REACH (For Korea-REACH). The regulations apply to any company that will manufacture or import any chemical subject to registration at 1 t or greater on an annual basis. The registration process will include hazard evaluation and risk assessment of the chemical within 1–2 years of registration. For more information visit the link provided here: http://eng.me.go.kr/eng/web/index.do?menuId=167.

Summary of Global Regulatory Frameworks

As demonstrated, there are varying levels of legislation regarding the management of chemical safety and risk assessment throughout the world. It is expected that efforts like those of the United Nations and World Health Organization will be used to continue the development of chemicals regulations and regulatory approaches to risk assessment. The REACH model of the European Union has attracted some interest and duplication in Asia. Some countries maintain databases and inventories, while others do not. The reader is encouraged to research countries and their unique requirements.

Chapter 11
Skills Development and Resources for Risk Assessment

José A. Torres and Nilsa Rivera-Del Valle

Abstract This chapter focuses on the skills and resources that are important for a career in risk assessment. Risk assessment desirable skills are categorized under quantitative, qualitative, computational, analytical, integrative, and soft skills. Resources presented include training opportunities (workshops), databases, professional journals, references books and other useful links.

Keywords Risk assessment skills · Workshops · SOT RASS · Training opportunities

Student Learning Objectives

- Raise awareness of necessary skills to pursue a career as a risk assessor toxicologist
- Provide useful resources for self-preparation

Introduction

The subject matter of risk assessment is not normally taught at academic institutions. Although institutions are becoming more aware of such needs, it may require time to adjust and provide risk assessment courses in the undergraduate and graduate programs. A major challenge for students interested in risk assessment is that most risk assessor experts work for industry and government agencies compared to the number of risk assessors working for academia. This results in fewer opportunities for undergraduate, graduate students and other trainees to learn and be engaged in the topic of risk assessment. If you, the reader, find yourself lacking from classes or knowledge on risk assessment but have the passion and interest in learning more

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about this subject, the resources and information laid out in this book and chapter should suffice as an introduction or supplement to a graduate level course. Specifically, this chapter provides information on the skills that are critical for a career in risk assessment and provides useful resources for self-preparation in the field.

Desirable Skills for Risk Assessment

The desirable skills for pursuing a career in the field of risk assessment discussed in this section were collected in a survey conducted in 2013. Selected participants were active members of the Society of Toxicology (SOT) and the Society of Toxicology Risk Assessment Specialty Section (SOT RASS). This survey asked experienced risk assessors currently working in this field to identify essential quantitative and qualitative skills for graduate students, post-doctoral trainees and early career professionals to better prepare for a career in risk assessment. The actual request submitted for the survey was: "*we hope you can provide us with what you think are the must have quantitative and qualitative skills for risk assessment. Another way to see this question is: if you were hiring a new member for your team what are the quantitative and qualitative skills that a job candidate must have to be a good candidate for the types of risk assessment positions you rely upon*." The following sections summarize the information collected from the survey, with the main goal that this information can aid in preparing students and other trainees in these areas.

Risk Assessment Quantitative Skills

Survey responses in the quantitative skills demonstrate a preference for qualified individuals with basic and advanced biostatistics understanding. To pursue mathematically demanding problems and modeling scenarios in risk assessment, the majority advised calculus I & II, graduate level statistics and probability courses. In addition, introductory courses in environmental engineering and kinetics are highly recommended. Even when knowledge on these areas is highly recommended, it is important to note that coursework is not sufficient or necessarily required to secure a job opportunity. Rather, these skills will prepare the applicant to compete for a job opportunity. Hiring managers expect the applicant to have the ability to apply acquired advanced mathematical knowledge to solve toxicology and epidemiology problems normally present in risk assessment.

Risk Assessment Qualitative Skills

In order to be a toxicologist risk assessor a strong knowledge in toxicology, biology, chemistry, epidemiology and public health is required. Other courses mentioned

as an advantage in the field of risk assessment include biochemistry, pathology, pharmacology, physiology, cellular biology, and cancer biology. An important aspect highlighted by the majority of survey participants is an understanding of epidemiological principles such as odds ratios, relative risk, risk ratios, rate ratios, and prevalence ratios; also to understand the distinction between hypothetical and real risk, and the knowledge of different public health issues.

Risk Assessment Relevant Computational Skills

Computational toxicology skills are an exciting area where growth is foreseen in the future. It came as no surprise that survey results indicated the need for applicants with computational skills. Acquiring computational skills to support risk assessment development sets an applicant apart from the rest of the pack. Again, it is important to note that knowledge in this area is not sufficient or necessarily required to secure a job opportunity, but computational toxicology skills may induce a hiring manager to further consider your credentials. Computational toxicology skills were reported as very important in the survey. Survey participants suggest becoming familiar with the U.S. Environmental Protection Agency (EPA) benchmark dose response (BMDS) software and ProUCL software. Both are available free at http://www.epa.gov/ncea/bmds/ and http://www.epa.gov/osp/hstl/tsc/software.htm *respectively*. BMDS is used to derive benchmark dose values for risk assessments and ProUCL is a statistical software package for analysis of environmental data sets with and without non-detect observations.

Statistical software to be familiar with includes the statistical packages R: http://www.r-project.org, MatLab: http://www.mathworks.com/products/matlab/ and SAS: https://www.sas.com/en_us/software/analytics/stat.html. Computer modeling is used to predict the risk of different chemicals. Computer modeling programs normally used in physiologically based pharmacokinetics (PBPK) includes Berkeley Madonna (http://www.berkeleymadonna.com) and acslX (http://www.acslx.com). Risk assessors may also face situations where basic testing and toxicological end point data is limited for certain compounds. In these circumstances, early evaluation of compounds may be subjected to a tiered screening process, using Quantitative Structure Activity Relationship (QSAR) models. Free QSAR software resources include ToxPredict-OPENTOX (http://apps.ideaconsult.net:8080/ToxPredict), CAESAR (http://www.caesar-project.eu/), and VEGA (http://www.vega-qsar.eu/). Advanced users may also employ the programmable OECD QSAR Toolbox (http://www.qsartoolbox.org/). Other commonly used commercial tools include TOPKAT (http://accelrys.com/mini/toxicology/predictive-functionality.html) and DEREK-Nexus (http://www.lhasalimited.org/products/derek-nexus.htm).

Thus, if computer programming is right up your alley, you might leverage that skill in the risk assessment field. Future in demand skills also include computer programming and computer modeling skills that use computational models of *in vivo* biology to interpret *in vitro* data. Another essential skill is the ability to calcu-

late dose response, use mathematical models, and perform low-dose calculations in computer applications. A must have skill is proficiency with Excel or spreadsheet-type risk assessment calculations. Therefore, computer programming can give you a competitive advantage in risk assessment areas where computational data mining and modeling are required (e.g. PBPK, low-dose risk extrapolation modeling).

Analytical Skill Set

Survey results indicated the importance of analytical skills. Analytical skills are important for risk assessors to evaluate multiple data sets including human epidemiology, animal toxicity tests, *in vitro* tests and results from molecular testing to discern a mode of action for a specific chemical. This skill is particularly important to assemble a description of the dose or time-related changes in effects. Analytical skills are necessary to analyze and understand: (a) how mode of action and weight of evidence fit into risk assessment analyses; and (b) how understanding mode of action of a chemical might help to determinant (likelihood of) causal or association between a specified chemical dose and a defined health effect.

Integrative Approach

As part of the analytical skill set, it is important to possess an integrative approach. An integrative approach refers to the ability to analyze complex data sets from different fields (eg. physical, chemical, physiological, *in vitro*, *in vivo*, epidemiological data, etc.) and present a clear and concise report or summary. An example of an integrative approach includes the analysis and synthesis of a set of preclinical data with whole animal systems perspective (e.g. integrating hematology, pathology and toxico-kinetic data) to develop an assessment of the compounds toxic potential.

To develop an integrative approach, the most commonly needed skills listed on the survey included knowledge and familiarity with the major classical toxicology evaluations (e.g. general toxicology, genetic toxicology, carcinogenicity, developmental and reproductive toxicology, and neurotoxicology) and the ability to develop hypotheses of mechanism of toxicity that can be translated into experimental approaches addressing human relevance of animal study findings. It is important to note that for risk assessment, the mechanistic studies are valuable when quantitative values are not available and in the absence of dose-response information.

Desirable Soft Skills

Soft skills can be defined as a cluster of qualities, habits, attitudes and social skills that define a qualified candidate for a specific position. During the survey, risk assessors described different soft skills needed or desired in a risk assessor. The most

common soft skills pointed to in the survey include excellent interpersonal skills, working well with other people and teams, professional dedication, be active in professional organizations, and run for leadership positions in such organizations. Other important skills include excellent writing and oral communication skills with the ability to articulate scientific arguments to non-technical audiences and a willingness to write manuscripts. A qualified candidate should feel comfortable working with a range of projects on different chemicals and answering a range of questions. In other words, a qualified candidate should be able to multitask and work under pressure.

In summary, survey results reveal a strong need for individuals prepared in the areas of mathematical, statistical, probability, computer modeling and programing knowledge. Equally important is to possess a strong background in toxicology, biology, chemistry and public health. The most requested and necessary skills highlighted in the survey were a strong preparation in epidemiology and advanced statistics that includes preparation in probability. The scientific and mathematical preparation allows the risk assessor to integrate complex problems found in risk assessment by using an interdisciplinary analytical approach. Current toxicology students generally finish their programs with strong preparation in biology, chemistry and toxicology. However, not all students are well prepared in advanced mathematical topics, computer modeling and public health. It is recommended that students interested in risk assessment engage in different electives and workshops to prepare them in the above mentioned areas.

To further identify desirable skills in risk assessment, a career development session was presented at the SOT 2013 annual meeting. Four risk assessors' experts from different sectors, including the industry and the government, were invited to share insights in the career development session. This session entitled "*Regulatory Science and Risk Assessment: Lessons for early-career scientist on what to expect and how to pursue this career path*" was sponsored by the SOT Post-Doctoral Association, and endorsed by the SOT Career Resources and Development Committee, SOT RASS and SOT Education Committee. The complete session was recorded and is accessible free of charge from the following link: http://www.toxicology.org/ai/crad/Seminars/riskassessment.asp.

During the session, invited experts identified several emerging needs and skill sets that will become important for future jobs in the field of risk assessment. Current trends point toward computational methods such as modeling, simulation, bioinformatics, physiological-based-toxico-kinetics, exposure modeling and biomonitoring. Also, validation of alternative methods and non-animal methods (structure activity relationships, threshold of toxicological concern [TTC]) are fast becoming valuable skills. Experts mentioned the need for individuals to work as part of a multifunctional team and integrate information from a variety of disciplines. It is worth noting similarities in the expert's commentaries and insights obtained in the career educational session with results obtained through the independent survey. Although independent of each other, expert risk assessors clearly agree on what is needed.

Practical steps to follow include becoming a member of different professional societies such as the Society for Risk Analysis and the SOT Risk Assessment Specialty Section, committing to life-long learning and connecting with thoughtful leaders. Once a member, ask for mentorship from a senior or seasoned risk assessor. Remember that these are busy professionals—take the view that you are building long-term relationships over time. The SOT also has a Mentor-Mentee database, available to its members. Another approach is to contact early career risk assessors as they are most likely solving current and relevant challenging problems and possess the necessary skills and time to provide information and orientation for the newcomer.

It is also advisable to understand how your research fits into the big picture and how your research might relate to risk assessment. Ask yourself the "So what?" question. Be resourceful, research the literature on risk assessment, talk to people and ask for training opportunities. If interested in the non-profit sector consider volunteering to demonstrate your passion. Volunteering for a non-profit can be a win-win scenario. Review the organizational mission statement and goals and think in terms of "does this organization's mission statement and goals align with my personal goals?" Do your homework and identify potential organizations of interest. Lastly, continue to develop your technical skills (translation across disciplines needed) and build your network of contacts.

In summary, the career development session insights coincide with the survey results. There is an evident need for well-prepared individuals with computational skills (modeling, simulation, bioinformatics), mathematics, and non-animal methods (structure activity relationship [SAR], PBPK, Thresholds of Toxicological Concern [TTC]) in the risk assessment field. The experts also recommended reading literature related to risk assessment, connecting with leaders, and asking for training opportunities. Both the survey response and experts in the career development session agree in developing skills that translate across disciplines and support teams with solving challenging problems.

Resources Available for in Depth Understanding of Risk Assessment

The ideal scenario for a student and other trainees interested in risk assessment is to gain experience under the mentorship of an expert risk assessor. Developing research projects involving risk assessment is also a great way to gain experience and knowledge in the field of risk assessment. As mentioned before, one of the challenges to gain experience and mentorship is that most risk assessors work outside academic institutions. Except for a few select institutions that possess outstanding faculty with expertise in this field, the newcomer may not have access to expert

mentorship and self-education is the first step to becoming familiar with this field and its opportunities.

In this chapter we covered most of the skills currently needed in the field of risk assessment. [Note, this chapter provides a long list of skills and information to prepare the reader for pursuing a career in risk assessment. The intent is to create an awareness of the many skills that will aid, rather than require, the newcomer to become familiar with all of the information presented in the chapter.

It is recommended that students and other trainees interested in risk assessment start their self-education by identifying a mentor outside their institutions. Look for professionals that hold a position within the interested sector, be it government, industry or academic. Furthermore, internships, if allowed by the student institution, are the perfect scenario to start acquiring knowledge and experience. In some instances, finding a mentor or completing an internship is not easy. In these instances, self-preparation is a good starting point while waiting for an opportunity in this field. Reading books and pursuing several educational venues may help the newcomer to understand the basic concepts of risk assessment while also searching for appropriate mentors, internships and other opportunities.

There are a number of excellent risk assessment training opportunities available. However, not all of these venues offer free courses. The reader may want to consider these courses as an investment in his or her career. The following sections will highlight some of the self-preparations materials available or suggested.

Reading Material on Risk Assessment

Four great resources to become familiar and/or read are: (1) National Research Council published Science and Judgment in Risk Assessment (1994) and Science and Decisions: Advancing Risk Assessment (2009), National Academy Press. Free download http://www.nap.edu/catalog.php?record_id=12209; (2) IPCS (International Program on Chemical Safety) 1999. Environmental Health Criteria 210: Principles for the Assessment of Risks to Human Health from Exposures to Chemicals, WHO, Geneva (http://www.inchem.org/documents/ehc/ehc/ehc210.htm); (3) Clewell, H.J. III, M.E. Andersen, and H.A. Barton. 2002. A consistent approach for the application of pharmacokinetic modeling in cancer and non-cancer risk assessment. Environmental Health Perspective, 110(1): 85–93. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1240697/; and (4) Estimating Exposure and Dose to Characterize Health Risk: The Role of Human Tissue Monitoring in Exposure Assessment (Sexton 1995). Free download from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1519013/.

Training Opportunities

Toxicology Excellence for Risk Assessment (TERA) teaches a 5-day intensive workshop on the subject of risk assessment. The TERA Dose Response Boot Camp provides emphasis in hazard identification and dose-response (http://www.tera.org/Global/Bootcamp/index.html). The Harvard School of Public Health offers a 4-day workshop: Analyzing Risk: Principles, Concepts and Applications. For more information visit: https://ecpe.sph.harvard.edu/programs.cfm?CSID=RISK0000&pg=cluster&CLID=1. The Latin American Risk Assessment Workshop (LARAW) provides a workshop more accessible for people living in Latin America or interested in risk assessment emerging issues in Latin America. Information on LARAW is accessible at: http://www.iutox.org/sprograms.asp. The Environmental Protection Agency (EPA) currently maintains a monthly webinar series of cumulative risk assessment technical panels and previously recorded videos are easily accessible at: http://epa.gov/ncer/cra/multimedia/webinars/2013/index.html. SOT RASS provides free monthly webinars for members. SOT RASS announcements are distributed through email to current members at http://www.toxicology.org/ISOT/SS/RiskAssess/downloads.asp. SOT provides free continuing education courses online for graduate students and post-doctoral trainees at http://www.toxicology.org/AI/ce/ce_video/index.asp.

Database and Useful Links

As mentioned before, an important skill is proficiency with database software used daily in risk assessment. The U.S. National Library of Medicine manages the Hazardous Substance Database: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB. This database provides peer reviewed toxicology information for approximately 5000 chemicals. The Royal Society of Chemistry offers the free database http://www.chemspider.com/. As described on their website, *"ChemSpider is a free chemical structure database providing fast text and structure search access to over 29 million structures from hundreds of data sources*." Familiarity with both databases would be an asset for a risk assessment career.

The EPA has a comprehensive database named Integrated Risk Information System or better known as IRIS (http://www.epa.gov/iris/index.html). This database is a great tool for hazard and dose-response identification. More specifically, as described on its website, "*EPA IRIS provides scientific support and rationale for the hazard and dose-response risk information in IRIS human health assessments. IRIS describes the health effects of individual substances for and contains descriptive and quantitative information on cancer and non-cancer effect for more than 540 chemical* substances*. The IRIS database contains information that can be used to support the first two steps (hazard identification and dose-response evaluation) of the risk assessment process."* Also, the EPA offers the National Center of Environmental As-

sessment (NCEA): http://www.epa.gov/ncea/. The EPA NCEA mission is to provide guidance about how pollutants may impact our health and the environment.

The Center for Disease Control or CDC provides free information about chemicals in Toxicological Profiles: http://www.atsdr.cdc.gov/toxprofiles/index.asp. The Agency for Toxic Substance and Disease Registry (ATSDR) is in charge of developing such toxicological profiles. The National Institution of Occupational Safety and Health (NIOSH) also provides chemical database information. This database is known as the NIOSH Pocket Guide to Chemical Hazards: http://www.cdc.gov/niosh/npg/. Other useful databases include U.S. government agencies that offer information on risk assessment such as the United States Department of Agriculture (USDA) http://www.fsis.usda.gov/wps/portal/fsis/topics/science/risk-assessments and the Office of Environmental Health Hazard Assessment (OEHHA) from the state of California http://www.oehha.ca.gov/.

Links providing important information on international regulations such as European regulations on chemicals include: REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) http://echa.europa.eu/web/guest/regulations/reach/understanding-reach, European Center for Ecotoxicology and Toxicology of Chemicals http://www.ecetoc.org/overview, and International Program on Chemical Safety http://www.who.int/ipcs/en/. In Europe, Food Safety and related risk assessment is regulated by the European Food Safety Authority (EFSA: http://www.efsa.europa.eu/) and in the United Kingdom, risk management programs are regulated by the Health and Safety Executive (http://www.hse.gov.uk/risk/).

Emerging online databases and programs with emphasis in modular systems are currently in development; the logic behind these is to help share the risk assessment workload and available data, improve transparency, facilitate collaboration, and provide a systematic approach to literature review. For example, Health Assessment Workspace Collaborative (HAWC) https://hawcproject.org is developed at the University of North Carolina at Chapel Hill School of Public Health. ICF International, a consulting firm, is developing an online modular system with the name of Dragon http://www.icfi.com/insights/webinars/2014/recording-dragon-a-suite-of-tools-for-systematic-literature-review. Both of these emerging programs are currently free to use and only required user registration. Yet another emerging online program currently in beta version was created by the Alliance for Risk Assessment (ARA). This program provides dose-response information and methods http://chemicalriskassessment.org/methods.

Other Applications for Risk Assessment

The field of Risk Assessment is not only limited to environmental and health studies, it is also used in the food industry, pharmaceutical and medical device industry and in the consumer product industry to evaluate the safety of millions of food, drugs, devices and personal care products used every day. This process is commonly known as Safety Assessment. In some instances, depending on the industry and the

regulations, the Risk Assessment process is part of what is called Risk Management. It is important to note that risk assessment used to evaluate the safety of food, drugs, device and personal products has a different format when compared to the traditional Environmental and Human Health Risk Assessment. However, the toxicological principles used are the same. This book does not cover the topics of Safety Assessment and Risk Management related to the above mentioned industries, but if the reader is interested in learning more about these topics and different regulations in these areas the following websites are recommended:

- **Pharmaceutical**: Labcompliance is a private organization offering information and training about the regulations that govern the Pharmaceutical & Medical Device industry. Some information is free of charge and additional information is available with an enrollment fee. http://www.labcompliance.com/tutorial/risk/default.aspx?sm=d_a
- **Food Safety**: World Health Organization (WHO), as described on their website: *"WHO is the directing and coordinating authority for health within the United Nations system*." http://www.who.int/topics/en/. An FDA related website is: http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/
- **Personal Care Products:** Personal Care Products Council, (previously known as the Cosmetic, Toiletry and Fragrance Association) is the leading national trade association that advocates on scientific, legal, regulatory, legislative and international issues related to the personal care industry. http://www.personalcarecouncil.org/category/science-safety.

The current Internet environment, with easy and free information available, allows the student or trainee to obtain valuable information. Table 11.1 shows a collection of online platforms providing free courses, in particular statistics, mathematics and biological sciences. These platforms are recommended not only for students but also for professionals who want to refresh some of the basic concepts and skills previously mentioned.

The Online platform Miriadax provides all its courses in Spanish. The other platforms provide most of their courses and educational material in English. This is not a comprehensive list, but rather a great place to begin shaping your skills free of cost and adjustable to your personal schedule. If you are aware of other resources, please feel free to contact the editors or authors for inclusion in future editions of this book.

Table 11.1 Online platforms offering free courses

Online platforms	
https://www.coursera.org	https://www.edx.org
http://oyc.yale.edu	http://www.saylor.org
https://www.open2study.com	https://www.udacity.com
https://www.canvas.net	http://online.stanford.edu/courses
http://oli.cmu.edu	http://alison.com
https://www.miriadax.net	https://iversity.org/courses

Table 11.2 Recommended books on the field of risk assessment

Human and Ecological Risk Assessment: Theory and Practice Dennis J. Paustenbach (Editor). 1592 pages Publisher: WileyInterscience 1 edition (May 14, 2002) ISBN-10: 0471147478	*Toxicity and Risk* Paul Illing. 168 pages Publisher: Taylor and Francis CRC ebook; 1 edition (April 16, 2007) ISBN-10: 0415233712	*Calculated Risks: The Toxicity and Human Health Risks of Chemicals in our Environment* Joseph V. Rodricks. 358 pages Publisher: Cambridge University Press; 2 edition (December 4, 2006) ISBN-10: 0521788781
Toxicological Risk Assessment of Chemicals: A Practical Guide Elsa Nielsen, Grete Ostergaard, John Christian Larsen Hardcover: 448 pages Publisher: CRC Press; 1 edition (February 21, 2008) ISBN-10: 0849372658	*Risk Assessment of Chemicals: An Introduction* C.J van Leeuwen (Editor), T.G. Vermeire (Editor) Hardcover: 688 pages Publisher: Springer; 2nd edition (October 23, 2007) ISBN-10: 1402061013	*Risk Assessment for Environmental Health* Mark G. Robson Paperback: 664 pages Publisher: Jossey-Bass; 1 edition (February 20, 2007) ISBN-10: 1118424069

Reference Books

Books are an essential part of professional training and education. The books presented on Table 11.2 focus on the basic building blocks of risk assessment. Target audiences are graduate students, post-doctoral trainees, early career scientist and other professionals that may benefit from a first time exposure to this subject. This book is not intended to be a comprehensive treatment on risk assessment, but rather an overview and introduction to preparing for a career in a complex and fascinating field. We are aware of many other excellent authors with outstanding books. The reader is encouraged to pursue additional subject matter texts for finding different approaches and perspectives to learning risk assessment.

Professional Journals

Professional Journals that publish different topics related to risk assessment are another excellent source of information. Examples of these journals include but are not limited to:

- International Journal of Risk Assessment and Management
- Human and Ecological Risk Assessment: An International Journal
- Integrated Environmental Assessment and Management
- Risk analysis: an official publication of the Society for Risk Analysis
- International Journal of Toxicology
- Journal of the American College of Toxicology
- Regulatory Toxicology and Pharmacology
- Toxicological Sciences

Summary

This chapter presented information on building basic stepping-stones toward preparing for a career in toxicological risk assessment. These basic steps are designed to help educate and prepare readers interested in the possibility of pursuing toxicological risk assessment as a profession. This is by no means an exhaustive list and does not make the reader an expert. Students interested in pursuing this career path are encouraged to find a mentor in the field of toxicological risk assessment. Become a member of professional organizations such as the Society of Risk Analysis and the Society of Toxicology Risk Assessment Specialty Section (SOT RASS). The SOT RASS organization possesses a cadre of excellent risk assessors. A possible option, if your program allows it, is to consider adjusting your research project to use the tools and build the required skills mentioned in this chapter to start your career on the risk assessment track.

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https://www.sas.com/en_us/software/analytics/stat.html
http://www.berkeleymadonna.com
http://www.acslx.com
http://apps.ideaconsult.net:8080/ToxPredict
http://www.caesar-project.eu/
http://www.vega-qsar.eu/
http://www.qsartoolbox.org/
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http://www.inchem.org/documents/ehc/ehc/ehc210.htm
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http://www.toxicology.org/AI/ce/ce_video/
http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB
http://www.chemspider.com
http://www.epa.gov/iris/index.html
http://www.epa.gov/ncea

http://www.atsdr.cdc.gov/toxprofiles/index.asp
http://www.cdc.gov/niosh/npg/
http://www.fsis.usda.gov/wps/portal/fsis/topics/science/risk-assessments
http://www.oehha.ca.gov
http://echa.europa.eu/web/guest/regulations/reach/understanding-reach
http://www.ecetoc.org/overview
http://www.who.int/ipcs/en/
http://www.efsa.europa.eu/
http://www.hse.gov.uk/risk/
https://hawcproject.org
http://www.icfi.com/insights/webinars/2014/recording-dragon-a-suite-of-tools-for-systematic-literature-review
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http://www.who.int/topics/en/
http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/default.htm
http://www.personalcarecouncil.org/category/science-safety
https://www.coursera.org
http://oyc.yale.edu
https://www.open2study.com
https://www.canvas.net
http://oli.cmu.edu
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http://online.stanford.edu/courses
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Chapter 12
Case Studies Chapter

Sol Bobst, José A. Torres and Rhian B. Cope

Abstract This chapter provides four hypothetical case study examples of risk assessment as they apply to (1) policy, (2) development of a reference dose concentration, (3) exposure assessment and (4) analysis of a parasiticide case study. The case study examples are not necessarily comprehensive of all risk assessment questions and considerations a risk assessor will evaluate. The examples are meant to provide the opportunity for the student to understand the application of risk assessment through case study approaches.

Keywords Benchmark dose · BMD · Reference dose (RfD) · PBPK · Uncertainty factor · HED · Brominated flame retardants · Ethylene Glycol · Acrylonitrile · Neonicotinoid Parasiticide

Student Learning Objectives

- Learning applied risk assessment through case examples
- Applying the knowledge and concepts discussed throughout the book

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Case Study Example #1: The use of Brominated Flame Retardants in Furniture

This case study is an example of how risk assessment decisions and policy can impact consumer and regulatory decisions. It also shows how different kinds of risk management decisions may have to be weighed by stakeholders.

Historically, brominated flame-retardants have been added to furniture in order to help assist with slowing the progress of a home fire. Many accidental fires started in homes can gain force and size when igniting and consuming other materials, which can result in unfortunate casualties. Scientific studies have shown that brominated flame-retardants are lipophilic, bioaccumulative, and suspected of causing neurobehavioral effects and endocrine disruption (Dagani et al. 2002; Arias 2001). Some European countries have banned the use of Polybrominated Diphenyl Ethers (PBDEs) and Polybrominated Biphenyls (PBBs).

A regulatory guideline in Europe, known as the RoHS Directive, has set a legal limit of 1 g/kg for the sum of PBBs and PBDEs (2002/95/EC). An argument known as the precautionary principle (PP) has been the driver for the ban in Europe. The PP consist of 4 main clauses: (a) pursue preventive action in the face of uncertainty; (b) the proponents of an activity are responsible to demonstrate its safety; (c) explore the range of possible of alternatives that provide the best outcome with respect to harmful action; and (d) provide public involvement in making decisions (Gilbert 2005). Finding new chemicals to inhibit ignition of furniture, as an alternative to brominated flame-retardants, will require new research and production processes, likely to increase the cost of goods. Fully removing any retardants from furniture may mean more people will die in house fires without furniture that contains flame-retardants. There are studies that have been conducted by academic institutions and sponsored by industry (Birnbaum and Staskal 2004), and each research group may criticize or accuse the other research body of having an underlying agenda.

As the reader begins to embark on a career in risk assessment, the novice will encounter future situations where there are varying opinions and options on what is the "right" decision. Answering these questions will depend on using the foundations of risk assessment, as presented in the chapters of this book. We intend this book to be a useful "benchmarking resource" to the beginner, and well as the experienced risk assessor, The approach is similar for addressing the appropriate development of drugs, food, and cosmetic products, occupational settings, as well as questions that address environmental conditions in soil, groundwater, and air quality.

Case Study Example #2: Development of a Reference Dose Concentration

This case study example provides the opportunity for the reader to walk through some of the steps of a hypothetical risk assessment calculation. It is for demonstrative purposes only and is not an opinion or conclusion on any specific chemical. While the details of every risk assessment maybe different, the steps here, for determining a reference dose concentration, are similar for all reference dose calculations.

Dr. Science-Woman (SW) has been assigned to conduct an evaluation of ingredient GX, it is a glycol derivative that is being developed for heating and cooling systems. It gives off a sweet smell, similar to Ethylene Glycol. The EPA has a current Test Order out under TSCA requirements to determine Oral Toxicity of GX. Dr. SW works for a contract research organization (CRO) hired by an industry consortia. She noticed that an oral reference dose risk assessment had been published recently (Snellings et al. 2013).

Dr. SW starts the process by doing hazard characterization. She confirms that the substance is chemically similar to Ethylene Glycol, which has been characterized as having a number of adverse health effects ATSDR (2010). Specifically acute oral toxicity with target organ damage to the kidney. Using read across approaches and some QSAR modeling, Dr. SW finds a positive prediction that GX has an acute toxicity endpoint. She decides to do the following experiments:

- *In vivo* Dose-Response measuring kidney injury/induced nephropathy through dosing of GX taken by oral ingestion.
- *In vitro* treatment of kidney cells to determine MOA through Calcium Oxalate Formation
- PBPK Modeling to examine the interspecies variability of toxicokinetics and toxicodynamics for ingredient GX was measured against dosing of GX by modeling Oxalate per Liter (OX/L) formation.

Step 1

Dose Response Assessment

Dose Response Assessment Data (Fig. 12.1)

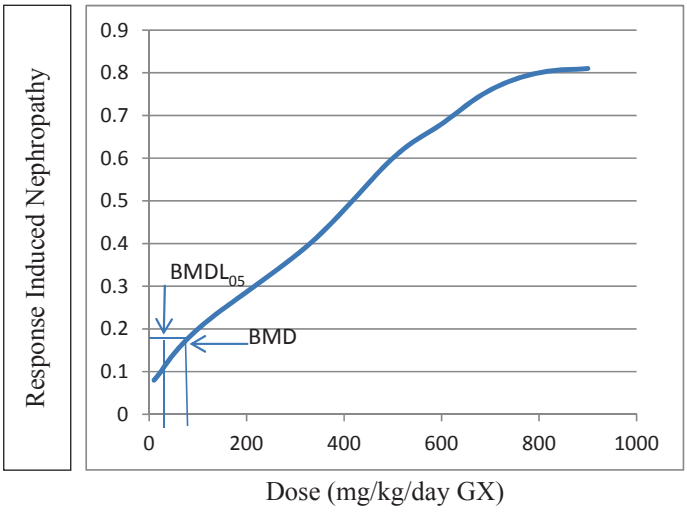


Fig. 12.1 *In vivo* measure of kidney injury with oral GX dosing

Step 2: RfD Calculation Set Up

To assess the impact of the nonlinear toxicokinetics of GX, RfD values were derived as the following

RfD1 = [BMDL05] HED/UF
Where: RfD = Reference Dose
BMD = Benchmark Dose
[BMDL05]HED = Benchmark Dose 95 % lower confidence limit; Human Equivalent Dose
Note: [BMDL05]HED is all one value determined from extrapolation

From the *In vivo* experiments and BMDL05[HED] determination, Dr. SW finds a value of 25. The default uncertainty factor is 100, this includes a default value of 10 for interspecies variability (rat to human) and intraspecies variability (within human). A straight, default calculation for the RfD would be equal to 0.25 mg/kg/day (25/100). Dr. SW knows this value would likely be considered too low to be reasonable to work with in an industrial setting. To determine if the uncertainty can be reduced, she plans to use a PBPK model that will compare human and rat toxicokinetics and toxicodynamics. The experiment does not address human variability, but it does address variability between rat and human. If the toxicokinetics and toxicodynamics are similar between rats and humans, this could reduce the intraspecies variability to parity or 1. Evidence that would support a reduction in uncertainty factors could mean that the reference dose concentration could be higher than the most conservative calculation that doesn't consider the PBPK model.

Based on a PBPK model of Calcium Oxalate Formation in the kidney from dietary dosing of GX in animals, the following PBPK Model and graph was determined. The graph shows minimal variability in toxicokinetics and toxicodynamics between rats and humans. This result is used to justify the reduction in Uncertainty Factors (Fig. 12.2).

Step 3: Determining Uncertainty Factors

Dr. SW evaluates the uncertainty factors as layed out in the table below (Table 12.1)

Final UF = UF Interspecies * Intraspecies = 10*1 = 10 (lower than 100)

Final Calculation
BMDL05—HED from Dose Response Curve In Rat Studies: 25 mg/kg/day

UF = 10

RfD = BMDL05(HED)/UF = 25/10 = 2.5 mg/kg/day

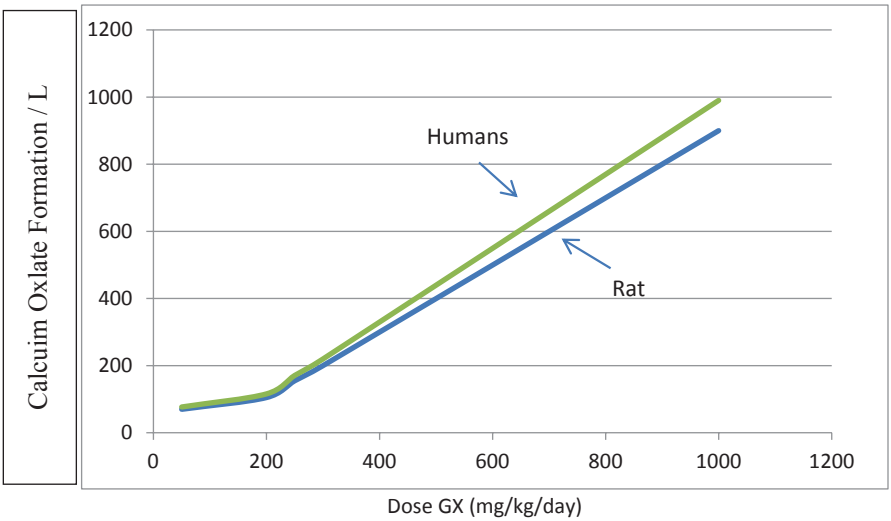


Fig. 12.2 PBPK model comparing human and rat calcium oxalate formation (OX/L) per oral dose of GX

Questions to consider:

1. Could a Human Equivalent Dose be derived another way, with the data provided? (Hint: Refer to Chap. 3 for review)
2. What are the advantages and disadvantages of using a conservative Bench Mark Dose Approach?
3. Could you, and how would you, develop a NOAEL or LOAEL value, based on this data?

Table 12.1 Collected uncertainty data

Normal uncertainty			
	ToxicoKinetics	*Toxicodynamics*	*UF*
Interspecies	Default 4	Default 2.5	10
Intraspecies	Default 3.16	Default 3.16	10
Interspecies uncertainty value for GX			*1*
PK reduction justification		PD justification	
Robust database		*In vitro* studies	
No variation in PBPK model		No variation in PBPK model	
Intraspecies uncertainty value			*10*
(No change from default)			

Case Study #3: Is the Accidental Release of Acrylonitrile a Reason of Concern?

Case Background

This is a hypothetical case developed for educational purposes and any resemblance to real-life is purely coincidental. "Rubber For You" is a company that works in the industry of textile fibers, synthetic rubber, and polymerized plastics, needs to provide maintenance work to several industrials tanks. The tanks require painting and mechanical maintenance when necessary. The work is scheduled to take place over 1 month period during the summer. The company "We Fix Anything" is hired to perform such maintenance. We Fix Anything pursue any kind of work (big or small) and their quotes are generally cheapest compared to competitors. Mr. Money, supervisor of We Fix Anything, sent Carlitos (painter) and Jamal (mechanic) to perform the maintenance contract at Rubber For You.

As Carlitos and Jamal worked the first week at the facilities of Rubber For You, they detected a pungent and noxious odor, but both assume that was a normal smell in this facility. This is the first time Carlitos and Jamal work on this kind of facility, but they are used to work in places with bad smells. During the second week of work, Carlitos felt the odor became overwhelming after having worked the whole day in such location. Jamal also felt the odor, although not in the same intensity as Carlitos. Mainly because Jamal's mechanical work required spending less time with each tank, unless changing valves, pipes and bolts became an issue, his responsibility is a quick visual inspection for mechanical problems. However they both reported the issue to "Rubber For You," supervisor Tom. Tom stated that he had been working at the facility for 40 years and not one person had died as a result of the smell, and that they need to stop wasting time and get the work done. But upon hearing this information, Tom hires an industrial hygienist from "We Detect Everything" and they found Air Monitoring levels of Acrylonitrile at 2.5 ppm.

By the end of week three, Carlitos and Jamal informed the strange issue to his supervisor at We Fix Anything, Mr. Money. Mr. Money responded that he is not concerned with any smell or odor; rather he is only concerned about finishing the job as soon as possible because he may land additional work in this facility.

The Rubber For You company wants you (an independent risk assessor) to: (a) evaluate the known toxicity hazards of Acrylonitrile; (b) to evaluate the evidence of exposure and effects of Acrylonitrile, particularly at 2.5 ppm; (c) search for any regulatory levels associated with Acrylonitrile; and (d) determine if the company should require the employees of "We Fix Anything" to wear respirators that would prevent inhalation at standard working condition level while completing the remaining work and any additional future job.

Chemical Summary: Acrylonitrile (CAS No. 107-13-1) (Fig. 12.3)

Introduction

Acrylonitrile is a reactive organic chemical used to make other chemicals such as polymerized plastics, synthetic rubber, and acrylic fibers. The fibers (acrylic and modacrylic) are primarily used in clothing and home furnishings. A mixture of acrylonitrile and carbon tetrachloride was used as a pesticide in the past; however, all pesticide uses have stopped. Acrylonitrile-butadiene-styrene resins are used in pipefittings, motor vehicles components, and large appliances. Copolymers of acrylonitrile are use in the production of beverages containers. Acrylonitrile is a commercially important industrial chemical that has been used extensively since 1940s with the rapid expansion of the petrochemical industry. The acrylonitrile manufacturing capacity was about 10 billions pounds (4,535,970 metric tons) in 1995 and United States accounts for 30 % of the world capacity.

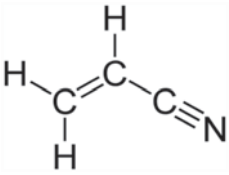
Chemical/Physical Properties

Acrylonitrile (CAS No. 107-13-1) is a clear, colorless to pale-yellow liquid with molecular formula C_3H_3N and molecular weight of 53.06. The yellowing color is upon exposure to light and indicate photo-alteration to saturate derivate. It is practically odorless, or with a very slight odor that may be describe as sweet, irritating, unpleasant, onion or garlic-like or pungent. Odor can only be detected above PEL. Boiling point of 77.3 °C and melting point of −82 °C. The specific gravity is 0.8004 @ 25 deg C, pH is from 6.0 to 7.5 (5 % aqueous solution), vapor density of 1.8 (Air = 1), Vapor pressure 109 mm Hg @ 25 °C. The Henry law constant is 1.38×10^{-4} atm cu m/mole @ 25 °C.

Chemical/Physical Properties

Molecular formula: C_3H_3N
Structural Formula: CH2 = CH–CN

Fig. 12.3 Acrylonitrile



Molecular Weight:	53.06
Color/Form:	Colorless to pale-yellow liquid. Yellowing upon exposure to light indicates photo-alteration to saturated derivates.
Boiling Point:	77.3 °C @760 mm Hg
Melting Point:	−82 °C
Corrosivity:	Attacks copper and copper alloys; attacks aluminum in high conc.
Specific Gravity:	0.8004 @ 25 °C/4 °C
Heat of Combustion:	1761.5 kj/mol @ 235 °C (liquid)
Heat of Vaporization:	32.65 kj/mol @ 25 °C
Octanol/Water Partition:	0.25
pH:	6.0–7.5 (5 % aqueous solution)
Solubility in water:	7 g 100 ml @ 20 °C
Surface tension:	26.6 dyn/cm @ 25 °C
Vapor Density:	1.8 (Air = 1)
Vapor Pressure:	109 mm Hg @ 25 °C
Relative Evaporation Rate:	4.54 (Butyl Acetate = 1)
Viscosity:	0.34cP @ 25 °C
Henry's Law constant:	1.38×10^{-4} at, cu m/mole @ 25 °C
Saturated conc. Air:	257 g/cu m @20 °C, 383 g/cu m @ 30 °C
Odor threshold:	21.4 PPM in air. Detection of acrylonitrile in water is $1.86 \times 10 + 1$ ppm; chemically pure
Other Properties:	Forms azeotropes with tetrachlorosilanes, water, isopropyl alchol, benzene, methanol, tetrachloride, chlorotrimethylsilane.

Occupational Exposure Standards

The OSHA 8 h TWA-PEL is 2 ppm; OSHA 10 ppm 15 min STEL; ACGIH 2 ppm 8 h TWA-TLV; NIOSH 1 ppm 10 h TWA-REL; IDLH 85 ppm. EPA RfC is 2e-3 mg/cu m (IRIS) based on rat 2-year inhalation studies where critical effects were degeneration and inflammation of nasal respiratory epithelium, hyperplasia of mucous secreting cells. The LOAEL is 20 ppm.

Suggested Approach to Solve This Case

Step 1

Based on what you learned in previous chapters begin by searching, collecting and organizing appropriate data. One suggested approach is presented here. But this is only a suggestion as there are many ways to organize and present chemical

Table 12.2 A possible approach to organize collected data

A) Chemical description and summary (**provided**)
B) Kinetic (ADME)
C) Metabolism
D) Biological half-life
E) Symptomatology
F) Toxicity data (Human and Animal)
General toxicology (acute/chronic; single exposure/multiples exposures)
Reproductive
Carcinogenicity
Genotoxicity and mutagenicity
Other related (e.g. Neurotoxicity, immunotoxicity etc.)
G) Exposure information (if available) in question
Occupational exposure standard (**provided**)
Routes, amount (concentration) and time
Affected proportion of the exposed number of individuals
H) Estimation of toxicity, hazard and risk
I) Risk evaluation
J) Conclusion of findings and references

information. The chapters of epidemiology and hazard identification provided the reader with excellence ideas and advance frameworks in how to classify human (epidemiological) data. Another simpler approach to classify epidemiological data is presented by Swaen (2006): (a) to identify agent in question; (b) classify the type of health effects in specific and non-specific manner; and (c) divide each health effect into acute/sub-acute/long-term. Then apply quality criteria for study design, quality of exposure data, and quality of effects data (Table 12.2).

Step 2

Now with the information collected and organized proceed to answer the following questions to help organize your ideas about this case study.

Question 1 Is there evidence of exposure, and if so, to what toxicant and what level or dose?

Question 2 What is known about the specificity toxicological effects that are associated with exposure to that toxicant (what dose is required to produce each effect; how long does it take to develop, etc.)?

Question 3 What other factors or conditions can possibly cause that symptom, and is it relevant in this scenario?

Question 4 What conclusions, if any, can be reached with the relevant information about the original question? Note: See if you can provide answer to the issues raised by the company that hires you.

Step 3

Proceed to organize the collected information into hazard identification, dose-response, exposure-assessment and risk characterization. Your goal is to practice how to present relevant information using the four steps of risk assessment, given that some information may be absent or not facilitated to you by your contract employer.

Case Study Example #4 An Example of a Human Health Risk Assessment: Human Health Risk Assessment of a Topical Neonicotinoid Parasiticide for Use in Cattle

Note: ***Please Read This Section Before Proceeding!***

This example is more extensive, designed to give the student more experience in risk assessment complexity and problem solving. It is deliberately very challenging and it *deliberately contains some potentially serious errors, omissions, paradoxical data and data that may or may not actually be required to perform the risk assessment.*

Introduction to the Example and the Student Questions and Challenges

The inclusion of errors, omissions, paradoxical and potentially superfluous data are every day challenges faced by risk assessment teams and regulatory reviewers in all areas of toxicological risk assessment. Learning to cope and deal with such challenges are as much part of toxicological risk assessment as performing common techniques such as dose response assessment! Some of the challenges raised by this example may also not have easy answers, or any answer at all, and will require perhaps uncomfortable compromises and trade-offs; again this is frequently part of the day-to-day real-world experience of being a practical toxicological risk assessor!

As you read through the example, carefully and thoroughly consider each step and assumption that is being made and carefully consider the paradoxes revealed by the data; ***critically evaluate the entire risk assessment for errors and omissions****!* As you become aware of the paradoxical information contained in the example, try to think about how a risk assessment team might handle such situations.

When you finish reading the example, attempt the student questions and challenges. *You may like to form a team with your classmates in order to do this*! Teams of risk assessors usually perform risk assessments like the one in the example: learning to operate within a risk assessment team is a BIG part of being a successful toxicological risk assessor!

Planning and Scoping

Scenario

You have been asked to perform a human health risk assessment on an external parasiticide designed for use in cattle grown for human consumption. The product is applied by "top lining" (manually jet sprayed down the center of the back of each animal) every 3 months. The target animal safety evaluation and the ecological risk assessment are not your responsibility (TASE and ecotox are performed by different section in your agency).

The product formulation is as follows:

- Active ingredient: a lipophilic neonicotinoid acaricide and insecticide; 1 g per liter of formulation;
- Excipients and solvents: hydrocarbons, C10-C12, isoalkanes, <2% aromatics (Cas. No. 64742–48–9); 999 g per liter of formulation.

The produce is applied without dilution at a rate of 0.1 L per animal (i.e. dose per animal = 1 g/L × 0.1 L/per animal/dose = 0.1 g/animal/dose = 100 mg/animal/dose) every 3 months.

Who/What/Where is at Risk?

The human populations at risk are:

- Workers involved in the manufacture of the product;
- Workers involved in transport of the product;
- Personnel involved in the response to spills and accidents involved with the product;
- Workers involved with loading of the jetting guns;
- Workers involved in applying the agent to the cattle;
- Workers involved in post-application handling of the cattle;
- Bystanders during the jet application process;
- Persons exposed due to transfer of residues on clothing into houses by workers;
- Exposure of persons re-entering the animal handling facilities;
- Consumers of residues in cattle tissues used for human consumption (dietary exposure).

The human populations of potential special concern are:

- Potentially highly exposed groups: workers in the manufacturing plant, responders to spills and accidents; contract applicators (loading, application, post-application handling) with regular, repeated daily exposures over long periods (several months to years); workers involved in loading, application and post-application animal handling;
- Potential high susceptibility groups: persons with multiple exposures to pesticides acting on the nicotinic nervous systems, children, pregnant women.

What are the Environmental Hazards of Concern?

The exposures of concern are:

- The neonicotinoid active ingredient;
- The solvent.

What are the Sources of the Environmental Hazards of Concern?

The sources of concern are:

- Direct exposures (manufacture, loading, application, bystanders);
- Indirect exposures (post-application animal handling, re-entry, residue transfer, dietary exposure).

What does the Body do with the Environmental Hazard and how is this Impacted by Factors such as Age, Race, Sex, Genetics, etc.?

Absorption

The available toxicokinetic data demonstrates that active ingredient has very low oral, and dermal bioavailability in cattle. In the initial dermal exposure toxicokinetic study *in cattle*, only 1 % of the topically ("toplined" by jetting) applied active ingredient is systemically absorbed. Thus the systemic exposure in cattle is:

$$\frac{1}{100} \times 100\, mg/animal/dose = 1\, mg/animal/dose$$

However, the initial dermal toxicokinetic study in cattle is complicated by the fact that the animals were not individually housed and were observed to mutually groom each other immediately following "toplining" of the test article. Thus the estimated dermal absorption of 1 % is probably actually due to a combination of dermal absorption and oral exposure.

Because of the confounding study design of the initially submitted dermal exposure toxicokinetic study in cattle, the study was repeated in animals that were individually housed in a manner that prevented mutual grooming and other sources of oral exposure. This study demonstrated that only 0.05 % of the topically applied dose is systemically absorbed, thus the actual systemic exposure (in the absence of oral grooming) is 0.5 mg/animal/dose.

At this point, a decision must be made on which study and which systemic exposure calculation is the most appropriate for the *practical real world* usage scenario. In the "real world" cattle would not be individually housed post-application. In reality, the cattle would be placed in a yard where they can mutually groom. Thus, in terms of systemic exposure *in cattle* the original study is the appropriate, even though it does not clearly distinguish between dermal absorption and oral absorption due to mutual grooming amongst animals.

A skin tape stripping study that utilized ^{14}C labeled active ingredient demonstrates that 48 h following toplining application in cattle, the neonicotinoid active is predominantly found in the outermost layers of the skin and on the hair coat (90 % of the recovered radioactivity is found in the first tape strip and hair sample). Smaller amounts of radioactivity were recovered from the deeper layers of the stratum corneum. No radioactivity was recovered from the sub-cornified layers of the skin. A subsequent histological autoradiography study of the skin utilizing ^{14}C labeled active and frozen sections demonstrated that virtually all of the radioactivity was present in the skin surface lipids and in the sebaceous glands at 48 h post-application. No radioactivity was present in the dermis or below the stratum corneum of the epidermis. A skin distribution study was also performed in cattle. This study demonstrated that following toplining, the active ingredient slowly distributed over the entire body surface of the animals over a 2-week post-application period.

In summary, the available dermal exposure toxicokinetic data in cattle demonstrates the following:

- Systemic absorption following dermal exposure in cattle is very low (circa 0.5 % of the toplined exposure);
- When animals are allowed to mutually groom following topline exposure, systemic absorption increases to about 1 % of the topical dose. This implies that, at least in cattle, some oral absorption of the active ingredient occurs;
- In cattle, the active ingredient predominantly partitions into the skin and hair surface lipids and into the sebaceous glands following topical exposure. The skin penetrance of the active ingredient is primarily limited to the skin surface and the outer stratum corneum. Very little absorption beyond the outer layers of the epidermis occurs;
- Following topline application, the active ingredient slowly distributes over the entre body surface of cattle over a 2-week post-application period.

As a follow up communication to these data, the company that produces the product has informed you that they intended to evaluate if they can take advantage of the oral exposure that occurs because of post-application grooming in order to treat gastrointestinal parasites. The company sees a commercial advantage of a topically applied product that can treat both external and internal parasites.

Because of the substantial anatomic differences between the skin of cattle and human skin, the company was requested to perform the skin absorption "triple pack" studies in order to provide an estimate of dermal absorption in humans. The skin absorption "triple pack" studies consist of the following measurements: (a) absorption through rat skin *in vivo*; (b) absorption through rat skin *in vitro*; and (c) absorption through human skin *in vitro*. The "triple pack" approach presumes, following normalization for % recovery, that if the ratio of *in vitro* rat skin absorption to *in vivo* rat skin absorption $\cong 1$, than *in vitro* human skin absorption is useful for extrapolating *in vivo* human skin absorption i.e. the results of the "triple pack" studies can be used to derive a human dermal absorption factor. "Triple pack" studies at different product dilutions were not required as the produce is not diluted before use. The rat component of the "triple pack" studies provided the following (simplified) data (Table 12.3):

The rat *in vitro* absorption: rat *in vivo* absorption ratio is 0.04:0.05 = 0.8. This suggests that the "triple pack" study methodology can make reasonable predictions regarding human dermal absorption *in vivo*. Given that the total *in vitro* human skin absorption over 72 h was 0.03 %, the predicted human *in vivo* skin absorption can be calculated:

$$\text{Predicted human } \textit{in vivo} \text{ skin absorption} = \frac{\textit{in vivo} \text{ rat absorption} \times \textit{in vitro} \text{ human absorption}}{\textit{in vitro} \text{ rat absorption}}$$

$$= \frac{0.05\% \times 0.03\%}{0.04\%} \cong 0.038\%$$

Oral exposure toxicokinetic studies in rats and mice have demonstrated that no detectable systemic absorption occurs in these species by this route of exposure. The company has provided a waiving argument regarding the provision of inhalation exposure toxicokinetic studies based upon the following key points:

- The active ingredient is non volatile and has a low vapor pressure at normal temperatures and pressures;
- The jetting equipment used to apply the product does not produce aerosols. Any droplets produced during the jetting process have a mass median aerodynamic diameter of much greater than 100 μm.

Table 12.3 Results of the skin absorption triple pack

Rat *in vivo* skin absorption study. Percentage absorption over time					
Time (h)	0	6	24	48	72
% absorption	0.00	0.00	0.00	0.00	0.05
Rat *in vitro* skin absorption study. Percentage absorption over time					
Time (h)	0	6	24	48	72
% absorption	0.00	0.00	0.00	0.00	0.04

Distribution

The available toxicokinetic studies in cattle and in rats indicate a volume of distribution equivalent to the blood volume of these species. This indicates that the active ingredient, once absorbed, has a small volume of distribution and is largely confined to the circulation. Distribution of the active ingredient to other tissues is minimal to undetectable.

Metabolism

The available toxicokinetic studies in cattle and rats demonstrate that no metabolism occurs.

Excretion

The available toxicokinetic studies in cattle and rats demonstrate that the major excretion pathway is movement into bile by energy-dependent mechanisms and elimination in feces. Substantial enterohepatic cycling occurs. Elimination at therapeutic doses appears to follow 1st order kinetics with a $T_{½}$ of 16 days.

What are the Health Effects?

The active ingredient is a neonicotinoid and thus the potential target is the nicotinic cholinergic nervous system (predominantly found in the CNS) of invertebrate parasites. In mammals, nicotinic cholinergic neuronal receptors are found in both the central and peripheral nervous systems. The binding of the neonicotinoid to the insect central nervous system nicotinic cholinergic receptors is essentially irreversible and binding triggers activation of the receptor i.e. the active ingredient acts as an irreversible nicotinic cholinergic agonist. The active ingredient has no to minimal effects on both mammalian muscarinic cholinergic receptors, even at extreme concentrations.

The active ingredient exhibits very substantial selective toxicity for insect and arachnids. Extensive comparative mode of action and susceptibility data in rodents, cattle, human, tick, and insect models has provided the following data:

- The acute, single exposure, oral (gavage) and dermal (occlusive) LD_{50} of the active ingredient in rats and mice is > 5000 mg/kg BW;
- The acute LD_{50} in various tick and insect parasites of cattle is in the 1–10 μg/kg BW range;
- *In vitro* studies of the comparative potency of the active ingredient on arachnid (cattle ticks, several different species), insect (buffalo fly, cat flea), rat,

mouse, cattle and human nicotinic cholinergic receptors indicates that insect and arachnid nicotinic acetyl choline receptors are approximately 1000 times more susceptible to the effects of the active ingredient compared with the equivalent mammalian receptors;
- *In vitro* studies have demonstrated that the active ingredient has a binding affinity for arachnid and insect nicotinic cholinergic receptors of approximately 1000 fold higher than for the equivalent mammalian (rat, mouse, bovine) receptors;
- *In vivo* studies have demonstrated that the active ingredient does not cross the intact adult blood-brain barrier in rats, mice and cattle. However, the active ingredient rapidly penetrates into the invertebrate central nervous system.

The results of the acute "6-pack" toxicity studies on the active ingredient indicate the following:

- Acute oral LD_{50} in rats and mice is > 5000 mg/kg BW;
- Acute occlusive and semi occlusive dermal LD_{50} in rabbits is > 5000 mg/kg BW;
- The company has supplied a waiving argument regarding the acute inhalation study based upon the low volatility and proposed usage of the active ingredient;
- The active ingredient is not an acute eye irritant;
- The active ingredient is not an acute dermal irritant;
- The active ingredient is negative in the mouse local lymph node assay and is thus not classified as a dermal sensitizer.

Studies in rats and mice have demonstrated the following data:

- 28-day repeat dose dermal and oral toxicity studies in rats and mice using the active ingredient have demonstrated no adverse effects (other than some evidence of mild skin irritation at the site of application in the dermal studies) in both rats and mice. The NOAEL and NOEL for both species is 1000 mg/kg BW/day, the highest dose tested[1];
- 90-day daily repeat dose dermal and oral toxicity studies in rats and mice using the active ingredient have demonstrated no adverse effects (other than significant skin irritation at the site of application in the dermal studies) in both rats and mice. The NOAEL and NOEL for both species is 1000 mg/kg BW/day, the highest dose tested.
- A near lifetime, daily repeat oral exposure (gavage) studies in rats and mice demonstrated in an increased incidence of fore-stomach mucosal epithelial hyperplasia, hyperkeratosis and inflammation in both species. The effects are dose-related (both incidence of the lesions and severity of the lesions increased with increasing dose). However, forestomach neoplasia was not observed. No other adverse effects were observed in the study.
- The chronic, daily repeat dermal exposure studies in rats and mice had to be terminated prematurely after 6 months due to unacceptably severe skin inflammation and ulceration at the site of application. Apart from severe skin

[1] Can you provide a scientific basis for the selection of the highest dose used in these studies? Hint: think about the dose of the final product that is used!

inflammation (and ulceration in some cases) in the mid (500 mg/kg BW/day) and high (1000 mg/kg BW/day) exposure groups, no other adverse effects were observed;
- Oral exposure prenatal developmental toxicity studies in rats and rabbits demonstrate no adverse effects. However, the highest dose tested in both studies (1000 mg/kg BW/day) was not maternotoxic;
- An oral exposure reproduction/developmental screening test in rats demonstrated that the active ingredient had no adverse effects except that transient muscular tremors were present in the live-born pups for a few hours (up to 3 h) immediately following parturition. All pups affected by the syndrome recovered completely by 24 h post-partum. They syndrome was not associated with any apparent anatomic abnormality and did not affect survival. The developmental NOAEL based on the muscular tremor syndrome was 250 mg/kg BW/day;
- Apart from muscular tremor during the first 24 h of life, no other adverse effects were observed in a developmental neurotoxicity study in rats;
- Acute, subchronic (90 day repeat daily exposure by gavage), and chronic (1 year repeat daily exposure by gavage) neurotoxicity studies in adult rats and mice demonstrate no adverse neuropathological, neurophysiological or neurobehavioral effects at dose up to 1000 mg/kg BW/day (gavage). Delayed (post-exposure) neurotoxicity is not present at up to 1000 mg/kg BW/day (gavage).
- The post-partum tremor syndrome is again observed in 2-generation reproductive studies in rats and mice. Again, the syndrome is only observed in the first 24 h of life and appeared to be completely reversible. The tremor syndrome was the only adverse effect observed and it did not affect reproduction or survival over 2 generations.
- The active ingredient had negative results in the BG1Luc estrogen receptor transactivation test, the H295R steroidogenesis assay, the Herschberger rat bioassay, and the rat uterotrophic bioassay.

Studies in cattle have demonstrated the following:

- A 10X duration, 0X, 1X, 3X, 5X therapeutic dose target animal safety study (i.e. repeat daily topline application for 10 days at 100, 300 and 500 mg/kg BW/day) in Hereford X Angus cross year old steers displayed no adverse effects;
- A 10X duration, 0X, 1X, 3X, 5X therapeutic dose target animal safety study (i.e. repeat daily topline application for 10 days at 100, 300 and 500 mg/kg BW/day) in pregnant cattle demonstrated a small, but statistically significant, increase (5% relative to the controls) in the incidence of mild arthrogryposis in calves born to cows exposed at the 5X (500 mg/kg BW/day) exposure level during days 40–70 of gestation.

The following genetic toxicology information has been supplied:

- Bacterial reverse mutation assay: negative in strains TA1535, TA100, TA102 and *E. coli* WP2 (with and without metabolic activation). Marginal positive response with strain TA98 and TA1537;
- Negative *in vitro* mammalian cell micronucleus test;

- Negative *in vitro* mammalian chromosome aberration test;
- Negative *in vitro* mammalian cell gene mutation test;
- Negative *in vitro* sister chromatid exchange assay in mammalian cells;
- Marginal positive in unscheduled DNA synthesis test in mammalian cells *in vitro*;
- Negative *in vivo* rodent erythrocyte micronucleus test;

Because of the marginally positive *in vitro* UDS test and the possibility of frame shift mutations in the bacterial reverse mutation assay (marginally positive results in strains TA98 and TA1537), the company was asked to perform a transgenic rodent somatic gene mutation assay. The company subsequently provided a sub-acute (28-day repeated daily oral exposure) study in the *lacI* mouse (Big Blue® mouse). The study demonstrated a small, but statistically significant, dose-related increase in mutations present in liver tissue. *In situ* RT-PCR data demonstrates that the *lacI* mutations predominantly occur in the Canals of Hering (intrahepatic bile ductules) of the intra-hepatic bile system. Sequencing data indicate that the predominant DNA lesions are frame shift mutations. Notably, based on the oral near-life time exposure studies in rodents, the active ingredient is *not* associated with increased incidences of cancers of the intrahepatic biliary system.

How Long Does it Take for the Health Effects to Occur?

The available data indicate that the active ingredient is a cumulative skin irritant in rats and mice under conditions of sub-acute to chronic ($\geq$28 days) repeated daily topical exposure. The active ingredient also produces a reversible post-partum tremor syndrome in rats during the first 24 h life. Exposure during pregnancy appears to produce the effect. Exposure between days 40–70 of pregnancy in cattle results in a small, but significant, increase in the incidence of arthrogryposis in calves. The mutations in the intrahepatic bile ducts appear to occur relatively quickly following exposure.

Known Regulatory Issues

Because of the positive transgenic mouse mutation study results, a preliminary legal evaluation of the relevance of the Delaney Clause of the US FD&C act was conducted. The Delaney Clause used to apply to pesticides in processed foods, but only when the concentration of a residue of a cancer causing pesticide increased during processing. Similarly, the Delaney Clause also applies to animal drugs in meat and poultry. Notably the Delaney Clause may not be applicable for the active ingredient based upon three exceptions:

- The active ingredient may fall under the *de minimis* exception if it is present in food at a concentration of less than 1 ppm;

- The active ingredient may fall under the threshold of toxicological concern limits for carcinogens of an exposure level of 1.5 μg per person per day (the active ingredient is not aflatoxin-like, and does not have N-nitroso- or azoxy- structures);
- In terms of its *pesticide use* the active ingredient is exempt from the Delaney Clause based on Title IV of the FQPA of 1996 (P.L. 104–107, Sec. 404). However, it is possible that the active ingredient may be considered as an indirect-food additive.

Notably, the presence of tissue residues of the active ingredient may represent issues pertaining to international trade.

Some concerns were also raised regarding the potential effects of the active ingredient on cattle egrets (*Bubulcus ibis*). Egrets are used as a biological control for cattle ticks and flies in some countries (notably Australia). It was decided that this should be an issue addressed in the ecotox evaluation.

Summary of the Planning and Scoping

At least some of the key issues arising from the planning and scoping phase of the evaluation are:

- Is the neo-natal tremor syndrome seen in rodents relevant to human health?
- Is the issue of an increased incidence of congenital arthrogryposis (crooked calves) in cattle relevant to human health?
- Is there sufficient selective toxicity with the active ingredient regarding the nicotinic cholinergic nervous system?
- Is the low level of frame shift mutations present in the intrahepatic bile ductules in rodents relevant and of concern to humans?

Hazard Identification

Evaluation of Data Quality

All supplied studies are GLP, and where relevant, consistent with current OECD guidelines. Accordingly all supplied studies are either Klimisch Score 1 or 2, which is acceptable and indicates that the data are reliable and of acceptable quality.

Summary of Identified Hazards

Based on analysis of the available data, the following hazards can be identified:

- The active ingredient is a fore-stomach irritant in both rats and mice following oral (gavage) exposure;

- A fully reversible, neonatal tremor syndrome in rodents. The syndrome does not appear to occur in cattle and is seemingly not associated with any longer-term adverse effects in rodents (does not affect reproduction, survival or neurological/neurobehavioral development in rodents);
- A small increase in the incidence of congenital arthrogryposis in calves that are exposed within the critical window of 40–70 days gestation (period of growth and development of the limbs). This critical window of exposure is approximately equivalent to approximately gestation week 4–8 in humans. Arthrogryposis did not occur in either the rat or the rabbit;
- There is sufficient evidence to classify the active ingredient as a weak frame shift mutagen within the intrahepatic biliary system following oral exposure. However, at least in rodents, the mutations are *not* associated with hepatic carcinogenesis following oral exposure.

Mode of Action and Human Relevancy Evaluation

The likely mode of action rodent neonatal tremor syndrome following oral maternal exposure is transient nicotinic overstimulation at the neuromuscular junction. Similar tremor syndromes are observed in a number of domestic mammalian species in the initial phases of nicotine poisoning and during the initial phases of excessive neuromuscular nicotinic stimulation associated with anticholinesterase poisoning. In the particular case of the rodent neonatal tremor syndrome, neuromuscular blockade does not appear to occur and the effect appears to be reversible. It is not known why this effect occurs in rodents but does not appear to occur in cattle or in adult rodents. It is assumed that the observed differences are due to structural differences in neonatal and adult neuromuscular nicotinic acetylcholine receptors. It is notable that the structure of embryonic and adult neuromuscular nicotinic receptors change during development: receptors are either the embryonic form, composed of α_1, β_1, γ, and δ subunits in a 2:1:1:1 ratio, or the adult form composed of α_1, β_1, δ, and ε subunits in a 2:1:1:1 ratio. It can be thus postulated that the rodent embryonic and adult forms of the receptor display different affinities and/or differing responsiveness to the active ingredient. In the absence of contradictory data, it is assumed that this effect is human relevant.

There are a number of examples of naturally occurring toxins that have nictotinic action in the peripheral nervous system that produce congenital arthrogryposis in cattle (e.g. anagyrine, anabasine, other piperidine alkaloids). The mode of action is assumed to be paralysis of the developing muscular system of the fetal limbs, resulting in joint fusions and other deformities. Such a mode of action is consistent with the neonicotinic mode of action of the active ingredient. In the absence of contradictory data, it is assumed that this effect is human relevant.

There is currently no data implying that the frame-shift mutations observed in the bile ductules in the oral transgenic mouse assay are not relevant to humans. Given that the transgenic mouse assay is a validated *in vivo* study, it takes precedence over the other available *in vitro* studies in terms of reliability for risk assessment

purposes. It should also be noted that *in vivo* rodent micronucleus test do not necessarily detect frame shift mutations, especially in the case of relatively weak mutagens.[2]

There is reasonable evidence that the active ingredient is a cumulative skin irritant.[3] This effect is likely to be relevant to humans.

Dose Response Assessment

Oral Repeat Exposure Dose Response Assessment[4]

- Acute oral LD_{50} in rats and mice is > 5000 mg/kg BW;
- Acute occlusive and semi occlusive dermal LD_{50} in rabbits is > 5000 mg/kg BW;
- 28-day repeat dose dermal and oral NOAELs and NOELs rats and mice is 1000 mg/kg BW/day, the highest dose tested;
- 90-day daily repeat dose dermal and oral NOAELs and NOELs in rats and mice is 1000 mg/kg BW/day, the highest dose tested.
- Near lifetime, daily repeat oral exposure (gavage) NOAELs in rats and mice was 250 mg/kg BW/day due to a statistically significant increase in the incidence of fore-stomach mucosal epithelial hyperplasia, hyperkeratosis and inflammation in both species. The relevant dose response data in both species is summarized in the following table (Table 12.4):

Table 12.4 Incidence of Forestomach Mucosal Lesions from the near lifetime repeat oral exposure (Gavage) studies in rats and mice

Species	0 mg/kg BW/day	100 mg/kg BW/day	250 mg/kg BW/day	500 mg/kg BW/day	1000 mg/kg BW/day
Rat	0/100	0/100	0/100	Total: 10/100	Total: 15/100
				Male: 5/50	Male: 7/50
				Female: 5/50	Female: 8/50
Mouse	0/100	0/100	0/100	Total: 9/100	Total: 16/100
				Male: 4/50	Male: 8/50
				Female: 5/50	Female: 8/50

[2] Student challenge: why would this be so? What do micronucleus tests *actually* measure? What are some of the key assumptions made when micronucleus tests are used to detect mutagens that non-clastogenic at low doses?

[3] Student challenge: what is meant by "cumulative skin irritant"? How does this differ from an acute skin irritant or a skin corrosive?

[4] Student challenge: since this product will be used topically and the only likely occupational exposure will also be dermal exposure, can you think of a reason as to why an oral dose response assessment and oral risk assessment is being performed?

- The chronic, daily repeat dermal exposure studies in rats and mice had to be terminated prematurely after 6 months due to unacceptably severe skin inflammation and ulceration at the site of application. Apart from severe skin inflammation (and ulceration in some cases) in the mid (500 mg/kg BW/day) and high (1000 mg/kg BW/day) exposure groups, no other adverse effects were observed. The incidence of skin inflammation after 6 months of exposure in both species are summarized in the following table (Table 12.5):
- The prenatal developmental and maternal NOEL was ≥1000 mg/kg BW/day;
- The developmental neurotoxicity NOAEL derived from the rat developmental neurotoxicity study was 250 mg/kg BW/day based upon neonatal muscular tremor during the first 24 h of life. The relevant data are summarized in the following table (Table 12.6):
- Acute, subchronic (90 day repeat daily exposure by gavage), and chronic (1 year repeat daily exposure by gavage) neurotoxicity studies in adult rats and mice demonstrate no adverse neuropathological, neurophysiological or neurobehavioral effects at dose up to 1000 mg/kg BW/day (gavage). Delayed (post-exposure) neurotoxicity is not present at up to 1000 mg/kg BW/day (gavage).
- Based on the results of the rat two-generation reproduction study, the F1 and F2 generation NOAEL is 250 mg/kg BW/day based upon the occurrence of the neonatal tremor syndrome within the first 24 h of life. The relevant data is summarized in the following table (Table 12.7):

Table 12.5 Incidence of skin lesions in the repeat dose dermal exposure studies in rats and mice

Species	0 mg/kg BW/day	100 mg/kg BW/day	250 mg/kg BW/day	500 mg/kg BW/day	1000 mg/kg BW/day
Rat	0/100	0/100	0/100	Total: 20/100	Total: 30/100
				Male: 9/50	Male: 15/50
				Female: 11/50	Female: 15/50
Mouse	0/100	0/100	0/100	Total: 21/100	Total: 32/100
				Male: 11/50	Male: 16/50
				Female: 10/50	Female: 16/50

Table 12.6 Incidence of neonatal muscular tremor in the rat developmental neurotoxicity study

Species	0 mg/kg BW/day	100 mg/kg BW/day	250 mg/kg BW/day	500 mg/kg BW/day	1000 mg/kg BW/day
Rat	0/20 litters affected	0/20 litters affected	0/20 litters affected	Incidence of litters with at least one affected pup: 6/20	Incidence of litters with at least one affected pup: 13/20
				Incidence of pups affected: 32/160	Incidence of pups affected: 70/156

Table 12.7 Incidence of neonatal muscular tremor in the rat two-generation reproduction study

Species	0 mg/kg BW/day	100 mg/kg BW/day	250 mg/kg BW/day	500 mg/kg BW/day	1000 mg/kg BW/day
F1 Rat	0/20 litters affected	0/20 litters affected	0/20 litters affected	Incidence of litters with at least one affected pup: 7/21 Incidence of pups affected: 30/152	Incidence of litters with at least one affected pup: 14/22 Incidence of pups affected: 73/155
F2 Rat	0/20 litters affected	0/20 litters affected	0/20 litters affected	Incidence of litters with at least one affected pup: 5/20 Incidence of pups affected: 34/148	Incidence of litters with at least one affected pup: 15/20 Incidence of pups affected: 77/145

Studies in cattle have demonstrated the following:

- The NOAEL for the 10X duration, 0X, 1X, 3X, 5X therapeutic dose target animal safety study (i.e. repeat daily topline application for 10 days at 100, 300 and 500 mg/kg BW/day) in pregnant cattle is 300 mg/kg BW/day based on a 5% increased incidence of mild arthrogryposis in calves born to cows exposed at the 5X (500 mg/kg BW/day) exposure level during days 40–70 of gestation. The relevant study data are summarized in the following table (Table 12.8):

The sub-acute (28-day repeated daily oral exposure) transgenic mouse *in vivo* mutagenesis study provided the following data (Table 12.9):

Table 12.8 Incidence of arthrogryposis in the cattle target animal safety study

Species	Control 0 mg/animal	1X 100 mg/animal	3X 300 mg/animal	5X 500 mg/animal
Cattle (pregnant cows)	0/40	0/40	0/40	Incidence of arthrogryposis: 2/40

Table 12.9 Results of the Transgenic Mouse *In Vivo* Mutagenesis Study

Species	0 mg/kg BW/day	100 mg/kg BW/day	250 mg/kg BW/day	500 mg/kg BW/day	1000 mg/kg BW/day
lacI mouse (Big Blue® mouse)	Incidence of animals with mutations 5/20	Incidence of animals with mutations 4/20	Incidence of animals with mutations 7/20	Incidence of animals with mutations 12/20	Incidence of animals with mutations 18/20

Techniques and Approach for Determination of Risk Threshold Values and Slope Factors

The techniques that are used for determination of risk assessment values (toxicological thresholds and mutation slope factors), and their justification, are as follows:

- 28-day repeat dose dermal and oral studies: The NOAEL technique will be used because there were no adverse or other effects at the highest dose tested (NOAEL and NOEL rats and mice is 1000 mg/kg BW/day). There is insufficient dose response data to enable the reliable use of bench mark dose techniques;
- 90-day daily repeat dose dermal and oral studies: The NOAEL technique will be used because there were no adverse or other effects at the highest dose tested (NOAEL and NOEL rats and mice is 1000 mg/kg BW/day). There is insufficient dose response data to enable the reliable use of bench mark dose techniques;
- Near lifetime, daily repeat oral exposure (gavage) studies: The NOAEL and will be used since there is insufficient dose response data to enable the reliable use of the bench mark dose technique;
- Reproduction/developmental toxicity screening test: The NOAEL will be used. In this particular study, the test article was administered to the mother and the effects were observed in the first 24 h of live *before* the pups started being dosed with the test article. This is an example of clustered data. The benchmark dose modeling technique assumes that the data are independent and is thus unsuitable for this data;
- Developmental neurotoxicity study: The NOAEL will be used. In this particular study, the test article was administered to the mother and the effects were observed in the first 24 h of live *before* the pups started being dosed with the test article. This is an example of clustered data. The benchmark dose modeling technique assumes that the data are independent and is thus unsuitable for this data;
- Two-generation reproduction study: Both the NOAEL and benchmark dose techniques will be used since there is sufficient dose response data to enable the use of both techniques. Ideally, nested benchmark dose analysis should be used for studies of this type of design. However for purposes of providing a somewhat simplified example, nested benchmark dose will not be used in this example;
- 10X duration, 0X, 1X, 3X, 5X therapeutic dose target animal safety study: Only the NOAEL technique will be used there insufficient dose response data to enable the use of the benchmark dose technique. Additionally, this study is an example of data clustering i.e. the test article is administered to the mother and the effects are observed in the offspring. The benchmark dose modeling technique assumes that the data are independent. However, clustered data is not independent and thus use of the benchmark dose approach is inappropriate for this data;
- *In vivo* transgenic mouse mutation assay: Unlike the other endpoints evaluated, mutagenesis is classically regarded (at least in regulatory toxicology terms) as a *non-threshold* response. Additionally, there is insufficient data to determine the shape of the low dose response curve (i.e. if the response is sub-linear or

supra-linear). In the absence of suitable low-dose data,[5] the default approach is to use linear low-dose extrapolation from the dose response curve point of departure (POD) in order to derive a mutagenesis "slope factor" (i.e. Δrisk/Δdose). A dose-related excess risk of 10^{-6} will be regarded as an acceptable level of excess risk in this case. The benchmark dose modeling software can be used to define the POD.

Derivation of Risk Threshold Values and Slope Factors

Using the EPA BMD modeling software for the oral near lifetime exposure study in rats where the critical effect was an increased incidence of fore-stomach mucosal epithelial hyperplasia, hyperkeratosis and inflammation (quantal data) resulted in the following modeled dose response curves (Fig. 12.4):

Basic visual inspection of the modeled curves demonstrates that none of them accurately reflect the available data. Additional statistical analysis is not required at this point. Since there are no other suitable models available, it is apparent that a BMD and BMDL cannot be accurately developed from the available rat data. Thus the rat NOAEL should be used in this particular case.

Using the EPA BMD modeling software for the oral near-lifetime exposure study in mice where the critical effect was an increased incidence of fore-stomach mucosal epithelial hyperplasia, hyperkeratosis and inflammation (quantal data) also resulted in no modeled curves that passed basic visual inspection. Thus the mouse NOAEL should be used in this particular case.

The developmental neurotoxicity NOAEL derived from the rat developmental neurotoxicity study was 250 mg/kg BW/day based upon neonatal muscular tremor during the first 24 h of life.

Exposure Assessment

Mixer/Loader Exposure Assessment

Since the product requires no mixing before use, a mixer exposure assessment is not required. Since the product is pre-packaged in a single use jet application system, no loader exposure assessment is required.

Applicator Exposure Assessment

There is currently no adequate modeling system for this form of exposure. The available USEPA PHED model data only applies to mechanically pressurized

[5] Student challenge: why is it so difficult to obtain accurate information regarding the shape of mutagenesis dose response curves at very low dose levels? Hint: read about the mega-experiments for mutagenesis and carcinogenesis.

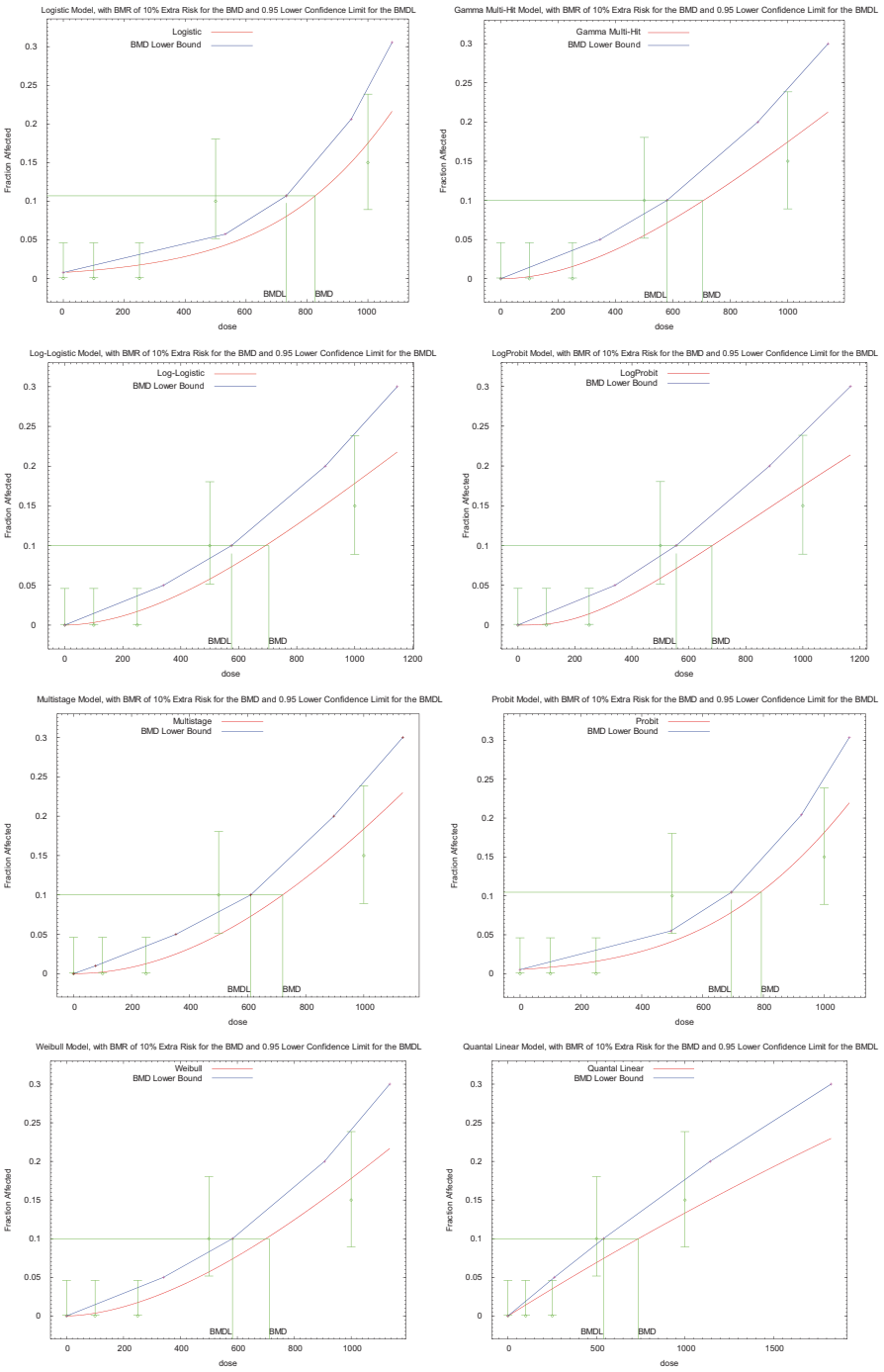


Fig. 12.4 Results of the bench mark dose modeling of the incidence of fore-stomach mucosal epithelial lesions in the oral (Gavage) near lifetime exposure study in rats

handgun sprayers. This is not appropriate for the pre-packages single use jet-application system associated with the product. The manufacturer has supplied an applicator occupational exposure study that appears to be well conducted and reliable (i.e. Klimisch score 2). This study indicates a dermal exposure level of 0. 6 μg/kg BW/animal treated for a 70 kg person provided that basic personal protective equipment (i.e. impermeable gloves, a face shield, long sleeve coveralls, boots and socks) is worn.

Bystander Exposure Assessment

There is currently no adequate modeling system for this form of exposure. The manufacturer has supplied an applicator occupational exposure study that appears to be well conducted and reliable (i.e. Klimisch score 2). This study indicates a dermal exposure level of 0.10 μg/kg BW/animal treated for a 70 kg person provided that basic personal protective equipment (i.e. impermeable gloves, long sleeve coveralls, boots and socks) is worn.

Post-Application Exposure Assessment

There is currently no adequate modeling system for this form of exposure. The manufacturer has supplied an applicator occupational exposure study that appears to be well conducted and reliable (i.e. Klimisch score 2). This study indicates a dermal exposure level of 0.06 μg/kg BW/animal treated for a 70 kg person 0–72 h post-application provided that basic personal protective equipment (i.e. long sleeve coveralls, boots and socks) is worn. The study assumes that treated animals will only be handled once in the 72-h post-application period (i.e. moved out of the cattle handling facilities and then not subsequently physically handled for 72 h).

Re-Entry Exposure Assessment

There is currently no adequate modeling system for this form of exposure. The manufacturer has supplied an applicator occupational exposure study that appears to be well conducted and reliable (i.e. Klimisch score 2). This study indicates a dermal exposure levels, while detectable (i.e. above the lower limit of detection with a signal to noise ratio of 3) will fall below the lower limit of quantitation (i.e. below a signal to noise ratio of 10) with the currently available assay techniques. Exposure associated with re-entry is regarded as negligible under these circumstances.

Transfer of Residues Into Households Exposure Assessment

The purpose of this exposure assessment is to address the transfer of residues from the workers into their place of residency (thus exposing other co-residents,

particularly children). There is currently no adequate modeling system for this form of exposure. The manufacturer has supplied an applicator occupational exposure study that appears to be well conducted and reliable (i.e. Klimisch score 2). This study indicates a dermal exposure levels, while detectable (i.e. above the lower limit of detection with a signal to noise ratio of 3) will fall below the lower limit of quantitation (i.e. below a signal to noise ratio of 10) with the currently available assay techniques. The assumptions made by this study are that workers will change out of their clothing and shower after use of the product and before returning to their place of residence. Provided that these assumptions are met, exposure associated with reentry is regarded as negligible under these circumstances.

Dietary Exposure Assessment

The purpose of this exposure assessment is to address the oral exposure of the general population through the consumption of edible tissues from the cattle treated with the parasiticide. The company has provided a tissue residue study based on data from 100 cattle and a withdrawal time of 2 weeks (i.e. a 2 week period between last application and sampling of edible tissues for residues). All tissue levels except for the kidney were below the limits of quantitation. The kidney contained 0.01 μg/kg of the neonicotinoid active ingredient. The available dietary survey data indicates that the 95th percentile level of consumption of cattle kidneys by the human population of interest is 0.001 kg cattle kidney/kg BW/day. Thus the 95th percentile limit of human exposure is:

$0.01\ \mu g/kg \text{ of cattle kidney} \times 0.001 \text{ kg of cattle kidney/kg BW/day} = 1 \times 10^{-5}\ \mu g/kg \text{ BW/day}$

$= 1 \times 10^{-8}$ mg/kg BW/day

Risk Characterization

As summary of the risk characterization and associated assumptions are presented in the following table (Table 12.10):

Student Questions and Challenges

1. The above risk assessment deliberately contains some potentially serious errors and omissions. Critically evaluate the entire risk assessment for errors, omissions and unstated assumptions. What would you, as a competent risk assessor, do differently? What additional analyses would you perform? How would you cope with some of the paradoxical data that has been supplied?

Table 12.10 Summary of the risk characterization and its assumptions

Population at risk	Key route of exposure	Key assumptions	Key effect	Point of departure	Uncertainty factors (If applicable)	Relevant risk value(s)	Exposure level	Risk ratio	Margin of exposure
Non-pregnant adult individuals in the general population	Oral (food)	Oral bioavailability in humans and rodents is equivalent[a] Withdrawal time of 2 weeks following last product usage Assume that rodent forestomach irritation is relevant to humans[b] Human body weight of 70 kg Consumption of cattle kidneys at ≤95th percentile level of the available dietary survey data	Gastric irritancy	NOAEL = 250 mg/kg BW/day daily life-time exposure (rat and mouse)	Interspecies = 10 Intraspecies = 10 Insufficient data to calculate CSAFs	ADI_{Oral} = 2.5 mg/kg BW/day daily life-time exposure	1×10^{-8} mg/kg BW/day	$2.5/1 \times 10^{-8} = 2.5 \times 10^{8}$	$250/1 \times 10^{-8} = 2.5 \times 10^{10}$
Pregnant women in the general Population and neonates in the first 24 h of life	Oral (food consumption by the mother)	Oral bioavailability in humans and rodents is equivalent Withdrawal time of 2 weeks following last product usage Human body weight of 70 kg Consumption of cattle kidneys at ≤95th percentile level of the available dietary survey data The rat neonatal neuromuscular tremor syndrome in rats is relevant to humans	Neonatal neuromuscular tremor in the first 24 h of life	NOAEL = 250 mg/kg BW/day[c]	Interspecies = 10 Intraspecies = 10 Insufficient data to calculate CSAFs	ADI_{Oral} = 2.5 mg/kg BW/day daily life-time exposure	1×10^{-8} mg/kg BW/day	$2.5/1 \times 10^{-8} = 2.5 \times 10^{8}$	$250/1 \times 10^{-8} = 2.5 \times 10^{10}$

Table 12.10 (continued)

Population at risk	Key route of exposure	Key assumptions	Key effect	Point of departure	Uncertainty factors (If applicable)	Relevant risk value(s)	Exposure level	Risk ratio	Margin of exposure
Non-pregnant workers, bystanders and household members dermally exposed to residue transfer to the houses	Dermal exposure	Assumes a worst case exposure scenario where an applicator was repeatedly exposed for up to 6 months Assume that the mode of action is solvent action/defatting effects on the skin Given the assumed mode of action, the interspecies and intraspecies uncertainty factors = 1 since humans are known to have equivalent sensitivity to these effects compared with rodents[d] Area of skin exposed in the rodent studies is equivalent to the area of skin exposed in humans[d] Assume 100 animals per day are treated[d] A single post-application handling exposure occurs at 0–72 h post-application Assume that workers use the personal protective equipment as indicated in the exposure assessment section	Cumulative skin irritancy	NOAEL = 250 mg/kg BW/day daily topical exposure over 6 months (rat and mouse)	Interspecies = 1 Intraspecies = 1 Insufficient data to calculate CSAFs	RfD_{Dermal} = 250 mg/kg BW/day daily topical exposure over 6 months	Applicator = 100 × 0. 6 μg/kg BW = 60 μg/kg BW/day = 0.06 mg/kg BW/day Bystander = 100 × 0.10 μg/kg BW = 10 μg/kg BW/day = 0.01 mg/kg BW/day Post application handling = 100 × 0.06 μg/kg BW = 6 μg/kg BW/day = 0.006 mg/kg BW/day Re-entry exposure = 0 Residue transfer to household = 0	Applicator = 250/0.06 ≅ 4166 Bystander = 250/0.01 = 25,000 Post application handling = 250/0.006 ≅ 41,666 Re-entry exposure = 0 Residue transfer to household = 0	Applicator = 250/0.06 ≅ 4166 Bystander = 250/0.01 = 25,000 Post application handling = 250/0.006 ≅41,666 Re-entry exposure = ∞ Residue transfer to household = ∞

Table 12.10 (continued)

Population at risk	Key route of exposure	Key assumptions	Key effect	Point of departure	Uncertainty factors (If applicable)	Relevant risk value(s)	Exposure level	Risk ratio	Margin of exposure
Non-pregnant adult individuals in the general population	Dermal exposure	Assumes a worst case exposure scenario where an applicator was repeatedly exposed for up to 6 months Assume that the mode of action is solvent action/defatting effects on the skin Given the assumed mode of action, the interspecies and intraspecies uncertainty factors = 1 since humans are known to have equivalent sensitivity to these effects compared with rodents[d] Area of skin exposed in the rodent studies is equivalent to the area of skin exposed in humans[d] Assume 100 animals per day are treated[d] A single post-application handling exposure occurs at 0–72 h post-application	Cumulative skin irritancy	NOAEL = 250 mg/kg BW/day daily topical exposure over 6 months	Interspecies = 1 Intraspecies = 1 Insufficient data to calculate CSAFs	RfD_{Dermal} = 250 mg/kg BW/day daily topical exposure over 6 months	Applicator = 100 × 0. 6 μg/kg BW = 60 μg/kg BW/day = 0.06 mg/kg BW/day Bystander = 100 × 0.10 μg/kg BW = 10 μg/kg BW/day = 0.01 mg/kg BW/day Post application handling = 100 × 0.06 μg/kg BW = 6 μg/kg BW/day = 0.006 mg/kg BW/day Re-entry exposure = 0 Residue transfer to household = 0	Applicator = 250/0.06 ≅ 4166 Bystander = 250/0.01 = 25,000 Post application handling = 250/0.006 ≅ 41,666 Re-entry exposure = 0 Residue transfer to household = 0	Applicator = 250/0.06 ≅ 4166 Bystander = 250/0.01 = 25,000 Post application handling = 250/0.006 ≅ 41,666 Re-entry exposure = ∞ Residue transfer to household = ∞

[a] Student Challenge: is this a reasonable assumption? Why is this assumption being made? Given the risk ratios and margins of exposure obtained, would such an assumption really matter in this case?

[b] Student Challenge: this would be a potentially controversial assumption: why? Given the risk ratios and margins of exposure obtained, would the controversy really matter in this case?

[c] Based on the results of the multigenerational study and supported by the developmental neurotoxicity study. This NOAEL is already lower than the 300 mg/kg BW/day for 10 days dermal exposure NAOEL for congenital arthrogryposis in cattle. Since

[d] Student Challenge: is this really a reasonable assumption?

2. Use the US EPA benchmark dose software package to derive the mutation slope factor for the active ingredient and then apply this slope factor within a risk characterization context.
3. One of the glaring omissions in the example is that a risk assessment of the solvents and excipients has not been performed. Would such a risk assessment be necessary?
4. Are there any studies that are missing from the data package? Are there additional studies that are needed to perform a thorough risk assessment?
5. Once you have completed challenges 1–4 above, write the risk communication section.
6. As the regulator in charge of whether or not this product should go on the market:
    a. How confident are you that the product has acceptable safety properties?
    b. Would you give the product final approval given the available data?
    c. How much overall confidence do you have in the risk assessment?
    d. If you approved the release of the product into the market place, how would you justify your decision to your management and to the non-technical general public?
    e. If you decided not to approve the release of the product into the market place, how would you justify your decision to the manufacturer? What additional data would you need to reverse such a decision?

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