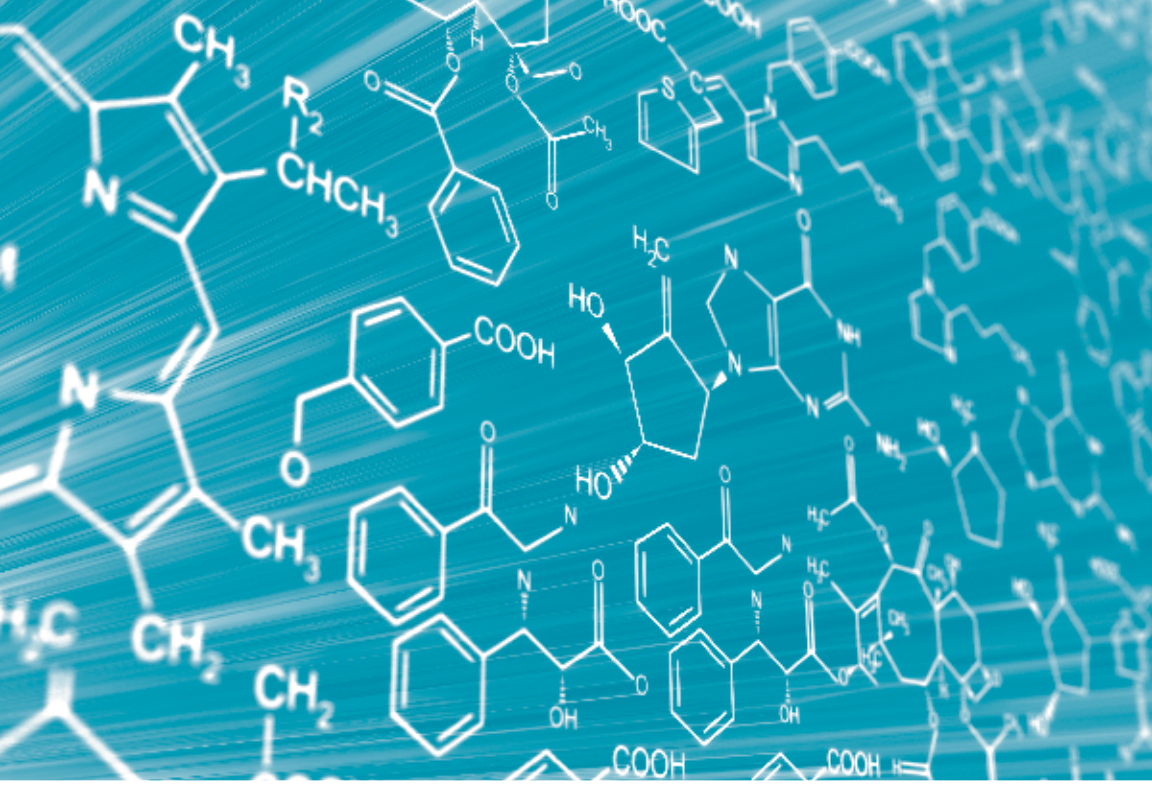

Integrating Emerging Technologies into Chemical Safety Assessment

The Expert Panel on the Integrated Testing of Pesticides

Council of Canadian Academies
Conseil des académies canadiennes

Science Advice in the Public Interest

INTEGRATING EMERGING TECHNOLOGIES INTO CHEMICAL SAFETY ASSESSMENT

The Expert Panel on the Integrated Testing of Pesticides

THE COUNCIL OF CANADIAN ACADEMIES
180 Elgin Street, Ottawa, ON Canada K2P 2K3

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This report was prepared for the Government of Canada in response to a request from the Minister of Health on behalf of the Pest Management Regulatory Agency via the Minister of Industry. Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors, the Expert Panel on the Integrated Testing of Pesticides, and do not necessarily represent the views of their organizations of affiliation or employment.

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Letter from the Chair

This report on the integrated testing of pesticides reflects the efforts and contributions of 15 panellists who brought a wealth of experience, knowledge, and perspective to this assignment. First and foremost, I am sincerely and deeply grateful to my colleagues on the Panel who contributed countless hours, days, and weeks so that our findings would be relevant, timely, and well-informed. Please know that your dedication to the task at hand did not go unnoticed. It was very much a pleasure and privilege to have had the opportunity to lead such a distinguished international group through many lively discussions and report drafts; and, to all of you, for having accorded me this opportunity, I am grateful. I am also indebted and grateful to Council staff for their support and assistance, and in particular for ensuring that we were in the right place at the right time and that we respected Council practices and policies. I want to specifically acknowledge and thank Renata Osika, Christina McMahon, and Michael Tyshenko.

I have reserved a very special thank you for Maria Trainer, Program Director at the Council with primary responsibility for this particular Panel. From the moment I first met Maria to respond to the invitation to serve as Chair of the Panel, her enthusiasm and spirit were infectious. In the year and a half that ensued to complete the work of the Panel, Maria brought rare talent, focus, discipline and, simply, boundless energy to complete a daunting task. I know my fellow Panellists join me in extending a heartfelt thank you to this outstanding young scientist.

Finally, my personal thank you to Elizabeth Dowdeswell, President of the Council, for your trust and confidence, both of which are very much appreciated.

Leonard Ritter
Chair, Expert Panel on the Integrated Testing of Pesticides

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Report Review

This report was reviewed in draft form by the individuals listed below — a group of reviewers selected by the Council of Canadian Academies for their diverse perspectives, areas of expertise, and broad representation of academic, industrial, policy, and non-governmental organizations.

The reviewers assessed the objectivity and quality of the report. Their submissions — which will remain confidential — were considered in full by the panel, and most of their suggestions were incorporated into the report. They were not asked to endorse the conclusions nor did they see the final draft of the report before its release.

Responsibility for the final content of this report rests entirely with the Expert Panel on the Integrated Testing of Pesticides and the Council of Canadian Academies.

The Council wishes to thank the following individuals for their review of this report:

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The report review procedure was monitored on behalf of the Council's Board and Scientific Advisory Committee (SAC) by **Dr. Judith Hall**, Professor of Pediatrics and Medical Genetics, University of British Columbia. The role of the report review monitor is to ensure that the panel gives full and fair consideration to the submissions of the report reviewers. The Board of the Council authorizes public release of an expert panel report only after the report review monitor confirms that the Council's report review requirements have been satisfied. The Council thanks Dr. Hall for her diligent contribution as review monitor.

Elizabeth Dowdeswell, President and CEO
Council of Canadian Academies

Executive Summary

INTRODUCTION

Pesticides are widely used in agriculture, industrial applications such as those to maintain hydro rights-of-way and, until recently, urban landscapes. The safety of pesticides has attracted enormous attention, particularly uses in urban and residential landscapes, and many provinces have already implemented, or are considering restrictions of these uses. The issue of pesticide safety, in general, and the assessment of pesticide safety by government authorities, such as Canada's Pest Management Regulatory Agency (PMRA) is a matter of health and environmental concern for many Canadians. Pesticide products typically comprise two components: an active ingredient that works against the target pest, and secondly a mixture of solvents and adjuvants in which the active ingredient is dissolved and which often aids in the intended action of the active ingredient.

In vivo:
Within a living organism. For example, toxicity tests conducted using animal models.
In silico:
Performed on a computer or by computer simulation.
In vitro:
In an artificial biological environment outside of a living organism.
Mechanistic endpoints:
Mechanistic endpoints are those that can be measured in assays that are designed to evaluate a specific cellular or physiological response. The precise mechanism in question depends on the level of biological organization at which the phenomenon is observed.

The active ingredients of pesticides are among the most stringently regulated compounds in commerce; the toxicological assessment (laboratory studies) of the active ingredient follows a regimen similar to the preclinical safety assessment of a prescription drug. Risk assessors use the toxicological data on pesticides to evaluate the ecological risks, the human health risks (including those from residues in foods), and risks arising from occupational and bystander exposures. This extensive evaluation of the active ingredients, however, contrasts with the data requirements for the other components of the final pesticide product. These formulants, which are added to pesticide products to improve their physicochemical properties, enhance their use, or increase their stability, are not typically subject

to a full battery of toxicity tests, and are often data-limited. As a result, the final pesticide product contains a combination of data-rich and data-poor chemicals.

The data-rich and data-poor nature of a pesticide formulation is a metaphor for the dichotomy that exists for most industrial chemicals. While there are some substances that have an enormous amount of data (e.g., pesticide active ingredients and pharmaceutical drugs), the vast majority of industrial chemicals are extremely data-poor. Indeed, recent estimates suggest that toxicity data are lacking for 87 per cent of chemicals on the market (reviewed in Hartung, 2009). Although regulatory agencies around the world are addressing these issues, the task of evaluating the safety of thousands of compounds cannot be fulfilled using the existing *in vivo* toxicity paradigm.

The international harmonization efforts among Organisation for Economic Co-operation and Development (OECD) member countries have led to the definition of standard data sets that must be submitted with all pesticide approval applications. As a result of these observations, the Panel concluded that pesticides make an excellent model group for developing a blueprint or framework for integrating new testing techniques into the existing approach.

"Today, we are neither effectively translating scientific discoveries into therapies nor fully applying knowledge to ensure the safety of food and medical products. We must bring 21st century approaches to 21st century products and problems…"

"Most of the toxicology tools used for regulatory assessment rely on high-dose animal studies and default extrapolation procedures and have remained relatively unchanged for decades, despite the scientific revolutions of the past half-century. We need better predictive models to identify concerns earlier in the product development process to reduce time and costs. We also need to modernize the tools used to assess emerging concerns about potential risks from food and other product exposures…"

Margaret A. Hamburg (Commissioner of the U.S. Food and Drug Administration) (2010), Advancing Regulatory Science. Science, 331 (6020), 987.

THE CONTEXT

Regulatory toxicology has traditionally relied on studies in laboratory animals coupled with estimates of human exposure to define the hazards and risks of chemicals. The current testing requirements for pesticide active ingredients prescribe an

extensive battery of tests that generate data on potential adverse effects for a wide range of endpoints, in different species, for different exposures, and over critical life stages and processes. Data from animal tests are used to identify potential adverse effects and develop dose-response relationships that are integrated with modelled (or measured) estimates of human exposure to serve as the basis for risk assessment for various pesticide use scenarios.

Over the last several decades, the testing of pesticide active ingredients has been extensive. As a result, these chemicals are among the most data-rich in commerce. Nonetheless, the current testing scheme for pesticides is expensive and time-consuming and, as such, cannot, on a practical level, be applied to the thousands of chemical entities which governments worldwide must now categorize. Consequently, there is a significant gap between need and capacity in toxicity testing.

Many of the current toxicology tests were developed over 30 years ago. As science has evolved in recent decades, so has our understanding of physiology; however, these advances have not been reflected in changes to the battery of toxicity tests that are required for regulatory decision-making (reviewed in Seidle & Stephens, 2009). Many of the standardized tests that are used in the existing toxicity testing battery, although state of the art at the time of their inception, "… have remained relatively unchanged for decades, despite the scientific revolutions of the past half-century. We need better predictive models to identify concerns earlier in the product development process to reduce time and costs. We also need to modernize the tools used to assess emerging concerns about potential risks from food and other product exposures" (Hamburg, 2010). Moreover, traditional toxicology protocols were not designed to generate (or incorporate) data pertaining to molecular mechanisms and signalling pathways.

The issues inherent in the current approach are therefore two-fold: to address the lack of toxicity data for the vast majority of industrial chemicals and to recognize that regulatory decisions must be based on the best available science. As a result, there is a need for new approaches that are more predictive, more reliable, faster, less expensive, and that provide mechanism-based, chemical-specific toxicity information in order to better inform human health risk assessment.

Building on advances in information sciences, biology (molecular, cellular, and systems), and reliable high-throughput screening assays pioneered in the drug discovery field, toxicology is about to transform into a science that incorporates knowledge of the biological pathways by which chemicals exert adverse health

effects. This will permit the evaluation of more substances and provide a better understanding of the intrinsic toxicological properties of different chemicals. Besides application to individual chemicals, these new approaches will also enable new methods for assessing the effects of combinations of chemicals and new ways of characterizing exposures.

IATA

Integrated Approaches to Testing and Assessment (IATA) describes a fundamental paradigm shift in the field of regulatory toxicity testing. This shift could move regulatory testing away from the one-size-fits-all prescribed battery of toxicity tests currently used to evaluate data-rich chemicals and towards a refined and focused testing strategy. This testing strategy could be tailored to the toxicity profile and intended use of the chemical in question and would be flexible enough to address the large number of chemicals with little (or no) toxicity data.

IATA adopts a hypothesis-driven approach that can incorporate new scientific advancements into the existing toxicity testing system in a transparent and scientifically credible manner. As such, it relies on a range of tools and techniques (*in vitro*, *in vivo*, and *in silico*) in order to focus testing resources on the toxicity endpoints of concern. Its strength lies in the breadth of information that is used to understand the toxicological profile of a chemical; ultimately, the collective information can more reliably inform a regulatory decision.

IATA: A tiered approach to data gathering, testing, and assessment that integrates different types of data (including physicochemical and other chemical properties as well as *in vitro* and *in vivo* toxicity data). When combined with estimates of exposure in an appropriate manner, the IATA provides predictions of risk. In an IATA, unsuitable substances are screened out early in the process. This reduces the number of substances that are subjected to the complete suite of regulatory tests. Plausible and testable hypotheses are formulated based on existing information and/or information derived from lower tier testing and only targeted testing is performed in the higher tiers. Failure to satisfy the toxicity requirements at a lower tier typically precludes further testing at a higher tier.

THE QUESTION

In May 2009, the Government of Canada, through the Pest Management Regulatory Agency (PMRA) of Health Canada, asked the Council of Canadian Academies to appoint an expert panel to answer the question, "What is the

scientific status of the use of integrated testing strategies in the human and environmental regulatory risk assessment of pesticides?" The charge to the Panel was further specified in a series of sub-questions:[1]

- What is the state of the science of the tools and data sources associated with integrated testing strategies?
- What is the current status of the use of integrated testing strategies for the risk assessment of pesticides, pharmaceuticals, industrial chemicals, and other chemical substances by regulatory agencies around the world?
- Could there be potential impacts on the public's perception and confidence in regulatory risk assessment and risk management decisions for pesticides if integrated testing strategies were implemented?

THE FINDINGS

What is the scientific status of the use of integrated testing strategies in the human and environmental regulatory risk assessment of pesticides?

> "We propose a shift from primarily *in vivo* animal studies to *in vitro* assays, *in vivo* assays with lower organisms, and computational modeling for toxicity assessments."
>
> Francis Collins (Director of the National Human Genome Research Institute and now Director of the US National Institutes of Health Toxicology) (2008). Transforming Environmental Health Protection. Science, 319 (5865), 906-907.

To date, aspects of computational toxicology (i.e., the use of alternative approaches to traditional animal testing) have primarily been used to support regulatory decision-making for data-poor chemicals such as pesticide formulants. Although the Panel is not aware of a complete set of alternative methods that could replace the entire testing paradigm today (even for data-poor chemicals), the state of the science is evolving rapidly. With the continued development of such tools and approaches, the Panel expects to see increased use of integrated testing strategies in decision-making, with an eventual adaptation to inform decisions involving data-rich chemicals. As such, these emerging technologies, integrated with existing data, are a pragmatic means by which new testing methods could be used to augment the regulatory paradigm and help bridge the transition to a hypothesis-driven approach to testing and assessment.

1 Although environmental and human health risk assessments share many of the same basic properties, they differ substantially in scope and underlying philosophy. As a result, the expertise needed to address the charge from the perspective of environmental risk assessment would be quite distinct from that of human health risk assessment. For this reason, given its expertise, the Panel chose to focus its assessment primarily on test methods that form the basis of human health risk assessment. While this report does not explicitly address creating toxicity pathways based on the biology of the target species of ecotoxicity testing, there is overlap and the report tries to draw linkages where possible.

What is the state of the science of the tools and data sources associated with integrated testing strategies?

Integrated Approaches to Testing and Assessment (IATA) represent a pragmatic approach that will move toxicology away from describing *what* happens towards explaining *how* it happens. There is no single IATA however. Fundamental to the use of any IATA is the existence of an adverse outcome pathway (AOP), which causally relates key events at different levels of biological organization to the *in vivo* endpoint of regulatory interest. Advances in numerous scientific disciplines are contributing to the rapid evolution of new and relevant tools. At the heart of this evolution are the fields of systems biology and computational toxicology.

IATA adopts and integrates tools from a wide variety of disciplines; these tools are all at different stages of readiness and are constantly evolving. Some of these tools use computational approaches to leverage existing toxicity data; others focus on generating new data using a variety of alternative approaches that harness rapid advances in systems biology. The acceptability and applicability of these tools for use in a regulatory context will be enhanced by the functional engagement of the international regulatory community and the execution of proof-of-concept studies that build confidence and familiarity in new approaches.

Over the past five years, significant research efforts have focused on developing new approaches and models for predictive toxicology and executing robust, proof-of-concept studies. These proof-of-concept studies have highlighted the importance of comprehensive and computable data and have shown the value of legacy data in the evolution of predictive toxicology.

As a result of these studies, IATA tools can now be used to make predictions about acute toxic endpoints. In the short term (one to two years) additional IATA approaches to evaluate critical local effects will likely be available. Non-animal replacement approaches to long-term endpoints (carcinogenicity, reproductive toxicity) are more challenging, and it is likely that it will be at least a decade before they are ready to be used in a regulatory context. IATA tools can also be used in a regulatory context to address the information gap for data-poor chemicals. Currently, regulatory decisions for data-poor chemicals are made based on little (or no) primary data.

What is the current status of the use of integrated testing strategies for the risk assessment of pesticides, pharmaceuticals, industrial chemicals, and other chemical substances by regulatory agencies around the world?

There are a number of examples of the use of components of IATA in a regulatory context for industrial chemicals and personal care products; however, there is no single example of a comprehensive hierarchical deployment of IATA in a regulatory context.

The Panel anticipates that the regulatory deployment of IATA strategies will vary depending on the type of chemicals in question and the nature of the decision-making process that the data are intended to inform. For data-poor chemicals, the lack of data supporting rational hypotheses for a plausible toxicological potential may be the impetus for a new approach. Data-rich chemicals are already subject to an extensive battery of toxicity tests; therefore establishing relevance may take longer and will be predicated on building and establishing trust in the new and novel methods. Although the adoption of IATA strategies might refine and streamline the testing of these chemicals as well as enhance the reliability of the outcome, the Panel does not anticipate a widespread deployment of IATA in the short term.

IATA is predicated on the use of all existing data in order to identify data gaps and ultimately to inform decision-making. As a result, the concept of an IATA that is grounded in an understanding of the biological mechanisms that explain toxicological effects could lead to a more efficient testing strategy so that not every endpoint for every chemical needs to be evaluated in an *in vivo* test.

The dynamic nature of IATA necessitates a new approach to test development and regulatory acceptance. Alternative methods (either testing or non-testing) typically target specific cellular or physiological responses and, as such, preclude validation with *in vivo* data by a one-for-one approach. The adverse outcome pathway (AOP) allows for the use of a suite of models or assays (and subsequent databases) that are designed to target particular steps along a specific pathway. Each assay/data set in an array of information would inform the next tier of the IATA or be used as part of an overall integrated testing strategy. The scientific justification of an alternative method should therefore focus on comparing the test outcome to what is known about the underlying biology as described in the AOP. As a result, the Panel believes that the scientific validation of an alternative

test method should be based on understanding the biological AOP or mode of action (MoA). Alternative tests would therefore be validated against mechanistic endpoints and not against a current *in vivo* protocol that may not be valid for predicting adverse outcomes in human populations.

Test development should be predicated on a functional collaboration between regulators and scientists to ensure that tests evolve to fit the needs of the testing paradigm. An evaluation and peer review of the assumptions, relevance, reliability, sensitivity, and specificity of alternative methods must occur prior to regulatory acceptance. This should be coupled with capacity-building initiatives within the regulatory community to develop comfort with the science underpinning the alternative tests and to build familiarity with the data that these tests produce.

> "The reason why new concepts in any branch of science are hard to grasp is always the same; contemporary scientists try to picture the new concept in terms of ideas which existed before."
>
> Freeman Dyson, 1958.

Could there be potential impacts on the public's perception and confidence in regulatory risk assessment and risk management decisions for pesticides if integrated testing strategies were implemented?

Yes. A major question that will be raised by implementing the new IATA tools in the regulatory system will be whether these changes enhance the ability to identify the most important risks to human health and environment or whether they compromise this ability in the interest of other social and economic values. The public will likely demand assurances that the new methods reduce overall uncertainties in the assessment of chemical risk and, where new uncertainties are introduced, that these will be handled in ways at least as precautionary as in the current system (see Chapter 5).

The risks associated with chemical pesticides are a particular worry to the general public, and changes in regulatory processes are sure to trigger concerns. Regulators will need to reassure the public that these changes are being made to provide more reliable assessments of health and environmental risks rather than to streamline processes and sacrifice safety for social or economic benefits.

While the strengths and weaknesses of the current system of chemical risk assessment are not widely understood by the general public, concerned stakeholders

are likely to evaluate any changes against this historical benchmark, regardless of its inherent limitations. The questions that regulators would need to address would likely include the following:

- Will the new IATA tools be used to supplement (and thus strengthen) the current system or to replace it?
- What scientific uncertainties in the current system of chemicals management are reduced by the implementation of new IATA tools? What new uncertainties are introduced by the use of these tools?
- How will the changes in the scientific uncertainties be handled in the regulatory process?
- Will the current "margins of safety" used in the *in vivo* toxicity testing regime be reduced?
- Will this lower safety standards with respect to certain kinds of chemicals?

The Panel believes that the new IATA tools can, and should only, be introduced into the regulatory system in a supplementary manner, and this can be done in such a way as to increase the ability of the system to identify more reliably the most significant risks, especially with respect to data-poor chemicals. If done in this way, the issues of public concern summarized above can be addressed in a way that maintains, and even strengthens, public confidence in the regulation of chemical pesticides.

Transparency is a critical component in the building of public confidence in the regulatory system as IATA tools are implemented. It is important that the use of new tools is explained as clearly and accurately as possible, and that the approaches for the handling of the changes in scientific certainty and uncertainty are made clear.

SUMMARY

> "All models are wrong, but some are useful."
>
> George Box, 1987.

Recent estimates suggest that toxicity data are lacking for 87 per cent of chemicals on the market (reviewed in Hartung, 2009). While the toxicological base supporting the safety of some chemicals, such as pesticide active ingredients, is extensive and has contributed significantly to our understanding of the toxicology of these products, on a practical level it cannot be applied to the tens of thousands of chemicals that regulatory agencies worldwide must now categorize. Consequently, there is a significant gap between expectation and capacity in toxicity testing, and an urgent need for new approaches that are more predictive, more reliable, faster, less expensive, and that provide mechanism-based, chemical-specific toxicity information in order to better inform human health risk assessment.

In May 2009, the Pest Management Regulatory Agency (PMRA) of Health Canada asked the Council of Canadian Academies to appoint an expert panel to answer the following question: "What is the scientific status of the use of integrated testing strategies in the human and environmental regulatory risk assessment of pesticides?" Although a complete set of alternative methods that could replace the entire current testing paradigm does not yet exist, the state of the science is evolving rapidly, and the Panel expects to see a global evolution toward the use of integrated testing strategies in decision-making, with the anticipation that this will better inform decisions for both data-rich chemicals and data-poor chemicals, over the next two to 10 years. The Panel expects that the regulatory deployment of Integrated Approaches to Testing and Assessment (IATA) will vary depending on the types of chemicals and the nature of the decision-making process that the data are intended to inform.

The potential risks associated with exposure to pesticides are already a particular worry for many people, and adoption of new IATA strategies in regulatory processes are almost certain to further underscore and exacerbate these concerns. Regulators must recognize the need to engage the public in meaningful dialogue in order to provide assurance that the new IATA approaches seek to reduce overall uncertainties in the assessment of chemical risk. Moreover, that these changes will provide more reliable assessments of potential risks to human health and the environment, rather than to simply streamline processes and sacrifice safety for social or economic benefits.

Contents

Common Abbreviations Used in this Report

AAAS	American Association for the Advancement of Science
AAFC	Agriculture and Agri-Food Canada
AcTOR	Aggregated Computational Toxicology Resource
ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism, and Excretion
AOP	Adverse Outcome Pathway
aPAD	Acute Population-Adjusted Dose
AR	Androgen Receptor
ARfD	Acute Reference Dose
BMD	Benchmark Dose
BPAC	Biological Pathway Activating Concentration
BPADL	Biological Pathway Altering Dose Lower Confidence Bound
BSE	Bovine Spongiform Encephalopathy
CAC	Codex Alimentarius Commission
CASRN	Chemical Abstract Services Registry Number
CCPR	Codex Committee on Pesticide Residues
CEPA	*Canadian Environmental Protection Act*
CFIA	Canadian Food Inspection Agency
CMP	Chemicals Management Plan
cPAD	Chronic Population Adjusted Dose
CSR	Chemical Safety Report
C_{SS}	Concentration at Steady State
DACO	Data Code
DCCA	(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid
DNA	Deoxyribonucleic Acid
DR	Dose Rate
DSL	Domestic Substances List
DSSTox	Distributed Structure-Searchable-Toxicity Database
EC_{50}	Median Effective Concentration
ECVAM	European Centre for the Validation of Alternative Methods
EDSP	Endocrine Disruptor Screening Program
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EFSA	European Food Safety Authority
ER	Estrogen Receptor
ES	Exposure Scenario
eSDS	Extended Safety Data Sheet
EU	European Union
FAO	Food and Agriculture Organization
FDA	*Food and Drugs Act*

FPT	Federal Provincial Territorial
GHS	Globally Harmonized System
GLP	Good Laboratory Practice
HPG	Hypothalamic-Pituitary-Gonadal
HPT	Hypothalamic-Pituitary-Thyroid
HPTE	2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane
HTRA	High-Throughput Risk Assessment
HPV	High Production Volume
HTS	High-Throughput Screening
IATA	Integrated Approaches to Testing and Assessment
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ILO	International Labour Organization
IOMC	Inter-Organization Programme for the Sound Management of Chemicals
IPCS	International Programme on Chemical Safety
IPM	Integrated Pest Management
ITS	Integrated Testing Strategy
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
kg	Kilogram
LC_{50}	Median Lethal Concentration
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
MAD	Mutual Acceptance of Data
mg	Milligram
MoA	Mode of Action
MoE	Margin of Exposure
MRL	Maximum Residue Limit
mRNA	Messenger Ribonucleic Acid
NAFTA	North American Free Trade Agreement
NGO	Non-Governmental Organization
NHANES	National Health and Nutrition Examination Survey (U.S.)
NIEHS	National Institute of Environmental Health Sciences (U.S.)
NIH	National Institutes of Health (U.S.)
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
NRC	National Research Council (U.S.)
NTP	National Toxicology Program (U.S.)

OECD	Organisation for Economic Co-operation and Development
PBPK	Physiologically Based Pharmacokinetic
PBT	PolyBrominated Terphenyl
PC	Physicochemical
PCPA	*Pest Control Products Act*
PCR	Polymerase Chain Reaction
PHAC	Public Health Agency of Canada
PMRA	Pest Management Regulatory Agency
ppb	parts per billion
ppm	parts per million
qHTS	Quantitative High-Throughput Screening
(Q)SAR	(Quantitative) Structure-Activity Relationship
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
RfD	Reference Dose
RNA	Ribonucleic Acid
SAR	Structure-Activity Relationship
SARA	*Species at Risk Act*
SDS	Safety Data Sheet
SMILES	Simplified Molecular Input Line Entry Specification
TG	Test Guideline
TGAI	Technical Grade of Active Ingredient
TR	Thyroid Hormone Receptor
TSCA	*Toxic Substances Control Act*
TTC	Threshold of Toxicological Concern
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
UV	Ultraviolet
vCJD	Variant Creutzfeldt-Jakob Disease
VTG	Vitellogenin
WHO	World Health Organization
WoE	Weight of Evidence
µg	Microgram
µM	Micromolar

1

Introduction to the Report

- The Charge to the Panel
- The Panel's Approach
- Defining the Problem that the Panel is Addressing
- Organization of the Report

1 Introduction to the Report

Pesticides are widely used in agriculture, industrial applications such as those to maintain hydro rights-of-way and, until recently, urban landscapes. The safety of pesticides has attracted international attention, particularly urban uses, and is the subject of hundreds of published scientific reports, the topic at many international conferences, the subject of expert reviews by respected international agencies such as the World Health Organization, and the legal responsibility of many national governments. The issue of the safety assessment of pesticides by government regulatory agencies, such as Canada's Pest Management Regulatory Agency (PMRA) is an important matter for many Canadians.

1.1 THE CHARGE TO THE PANEL

In May 2009, the Government of Canada, through the Pest Management Regulatory Agency (PMRA) of Health Canada, asked the Council of Canadian Academies to appoint an expert panel to answer the question "What is the scientific status of the use of integrated testing strategies in the human and environmental regulatory risk assessment of pesticides?" The charge to the Panel was further specified in a series of sub-questions:

- What is the state of the science of the tools and data sources associated with integrated testing strategies?
- What is the current status of the use of integrated testing strategies for the risk assessment of pesticides, pharmaceuticals, industrial chemicals, and other chemical substances by regulatory agencies around the world?
- Could there be potential impacts on the public's perception and confidence in regulatory risk assessment and risk management decisions for pesticides if integrated testing strategies were implemented?

1.2 THE PANEL'S APPROACH

To address the charge, the Council of Canadian Academies assembled a diverse group of leaders from Canada and the United States specializing in the life sciences, relevant aspects of computer science, ethics, law, risk assessment, and public policy.

The Panel met on six occasions between October 2009 and April 2011. During the deliberative process they considered the vast body of literature relevant to

their charge. In addition, they solicited input from a wide variety of stakeholder groups via consultations and written submissions. The Panel reviewed the evidence provided by these groups and incorporated that information into their deliberations. An assessment of the international status of issues relevant to the Panel's work was also completed and taken into consideration. As with all Council of Canadian Academies' reports, the Panel's draft report underwent a rigorous report review process by 11 anonymous peer reviewers whose expertise reflected that of the Expert Panel.

1.3 DEFINING THE PROBLEM THAT THE PANEL IS ADDRESSING

The field of toxicology is unique because it straddles two "worlds" that are almost diametrically opposed in fundamental philosophies and that operate under very different constraints. As a research discipline, basic toxicology is driven by rational analyses and technical judgments; it exists to expand our understanding of the relationship between exposure and effect by an iterative, hypothesis-driven process. Uncertainty is embraced and forms the foundation for subsequent research endeavours. In contrast, regulatory toxicology exists to facilitate the predictive certainty mandated by the legislative process in order to inform regulatory decision-making and safeguard public health. The issues that define the research questions for regulatory toxicology are often multidimensional and are driven by statutory and political processes.

Regulatory toxicology has played an important role in the management of chemicals and the protection of human and environmental health for several decades. In this sense, chemicals represent a broad spectrum of products that could be absorbed by a living system and pose an adverse risk to health; this definition includes, among others, pharmaceuticals, industrial chemicals, and agricultural products.

Data generated in regulatory toxicity testing are used to inform a risk assessment that must consider both human health and environmental factors. Although environmental and human health risk assessments share many of the same basic properties, they differ substantially in scope and underlying philosophy. As a result, the expertise needed to address the charge from the perspective of environmental risk assessment would be quite distinct from that of human health risk assessment. For this reason, given its expertise, the Panel chose to focus its assessment primarily

on test methods that form the basis of human health risk assessment. The Panel did, however, note that a similar effort is needed to re-evaluate the current testing tools available for ecotoxicology.[2]

1.3.1 What are Integrated Approaches to Testing and Assessment?

Integrated Approaches to Testing and Assessment (IATA) describes a fundamental shift in the field of toxicity testing. It moves toxicity testing away from the one-size-fits-all prescribed battery of toxicity tests that has been used for decades (introduced and discussed in more detail in Chapter 2), towards a refined and focused testing strategy; one that is tailored to the toxicity profile and intended use of the chemical in question. An IATA strategy uses a tiered approach; all of the existing data on a substance are compiled at the start of the testing process in order to evaluate what data gaps exist and what testing approaches would be most appropriate to elucidate the precise toxicological profile of that substance.

IATA relies on a multitude of tools and techniques from numerous scientific fields and disciplines. Its strength lies in the breadth of information that is used to understand the toxicological and exposure profile of a chemical; ultimately, this collective information is used to inform a regulatory decision.

1.3.2 Why Pesticides?

The active ingredients of pesticides are some of the most stringently regulated chemicals in commerce; the toxicological assessment (laboratory studies) of the active ingredient follows a regimen that is similar to that for the preclinical assessment for the safety of a prescription drug. Risk assessors use these data to evaluate the ecological risks, the human health risks (including those from residues in foods), and risks for occupational exposures that will arise if this novel chemical entity is registered in Canada for use as a pesticide. This extensive evaluation of the active ingredients in pesticides, however, contrasts with the data requirements for the other components in the final pesticide product. These formulants, which are added to pesticide products to improve their physicochemical properties, enhance their use, or increase their stability, are not typically subject to a stringent battery of toxicity tests and are often data-limited. As a result, the final pesticide product contains a combination of data-rich and data-poor chemicals.

2 The Panel notes there is an ongoing US EPA-sponsored National Research Council study, Human and Environmental Exposure Science in the 21st Century. The purpose of this study is to develop a long-range vision for exposure science as well as a strategy (with goals and objectives) to implement that vision over the next 20 years. Further information may be found at: http://dels.nationalacademies.org/Study-In-Progress/Human-Environmental-Exposure-Science/DELS-BEST-09-02

The data-rich and data-poor nature of a pesticide formulation is a metaphor for the dichotomy that exists for most industrial chemicals. While we have an enormous amount of data for some substances (e.g., pesticide active ingredients and pharmaceutical drugs), the vast majority of industrial chemicals are extremely data-poor. Indeed, recent estimates suggest that toxicity data are lacking for 87 per cent of chemicals on the market (reviewed in Hartung, 2009). Although regulatory agencies around the world are starting to address these issues, it is widely recognized that new regulatory testing requirements cannot be fulfilled using the existing *in vivo* toxicity paradigm.[3; 4]

As a result of these observations — coupled with the international harmonization efforts by Organisation for Economic Co-operation and Development (OECD)-member countries that have led to the definition of standard data sets that must be submitted with all pesticide approval applications — the Panel concluded that pesticides make an excellent model group to develop a blueprint or framework for the integration of new testing techniques into the existing approach.

The Panel chose to focus their report around three main "pillars" in order to define their undertaking and provide evidence-based, expert opinions to answer the main question and sub-questions in accordance with their mandate:

- Where possible, distinguishing between data-rich and data-poor chemicals;
- Where possible, populating with case studies to provide real-life context; and
- Where possible, discussing short-, medium-, and long-term options.[5]

1.3.3 Why Now?

Advances in understanding the fundamental processes that govern the physiological response to exposure have led to initiatives that seek to move toxicity testing away from observations of apical endpoints in model species and towards mechanistically based assays in human cells.[6] While these technologies are in their infancy, they speak to a need to ensure that regulatory toxicology is well positioned to take advantage of rapid advances in fundamental science; this will provide assurances that regulatory decisions are made on the basis of the best available scientific evidence.

3 An exhaustive list of relevant reports and legislative initiatives is not included here for reasons of brevity; however, many of these documents will be cited throughout this report.

4 The Panel acknowledges that for the active ingredients in a pest control product, throughput is not a bottleneck *per se*. Rather, besides the active ingredient, the other components in the formulation will need to be evaluated more thoroughly in the future. Nonetheless, the safety assessment of the active ingredients will benefit from tools that are based on a mechanistic understanding of both chemistry and biology.

5 In this regard, the Panel defines short term as zero to two years; medium term as approximately five years; and long term as approximately 10 years.

6 An apical endpoint is defined as an empirical observable outcome from an animal study.

The future of toxicity testing may well be an array of assays that are all performed *in vitro* and *in silico* with little or no need for animal tests. This vision, however, will only be realized by incremental advances in scientific knowledge and regulatory acceptance. It is a vision for the future but not one that will be realized in its entirety for many years to come. With this in mind, the Panel has chosen to focus its attention on scientific advances and regulatory changes that may be realized over the next decade.

The Panel believes that integrating new science into the regulatory process in an iterative fashion will facilitate the co-evolution of toxicity testing and regulatory acceptance — a co-evolution that will be necessary in order to meet the needs of new legislative mandates that will increase the toxicity data requirements for those data-poor chemicals that are currently not subject to extensive pre-market testing.

Furthermore, integration of new test methods that augment existing toxicity testing requirements would promote public acceptance of these alternative approaches.

1.4 ORGANIZATION OF THE REPORT

In order to provide sufficient context to adequately address the charge, the Panel describes and critiques the existing regulatory framework for pesticides in Chapter 2. Chapter 3 provides a comprehensive review of the state of science underpinning an IATA approach and profiles a number of examples of IATA relevant to regulatory toxicity testing. Chapter 4 illustrates where IATA, or components of an integrated strategy, are being used in a regulatory environment to inform risk assessments. Chapter 5 reviews public perceptions of risk and highlights the importance of communication and stakeholder engagement. Chapter 6 summarizes the Panel's findings and presents their conclusions regarding the evolution of IATA in the regulatory environment over the next decade. Each chapter is written in such a way that it can be read either as part of the complete report or as a stand-alone document.

Toxicology is an inherently interdisciplinary and technical endeavour. To assist the reader in navigating some of the specialized language used throughout the report, a list of key terms is included at the start of Chapters 2 through 5, and a more complete technical glossary may be found in Appendix A. A list of key abbreviations is provided on page xxiv.

2

The Current Approach to Regulatory Testing and Risk Assessment

- A Brief Introduction to Pesticide Testing and Regulation
- International Cooperation on Pesticides Regulation
- The Assessment and Management of Pesticide Risks
- The Existing Toxicity Testing System
- Critique of the Current Toxicity Testing System
- Addressing the Limitations: Integrated Approaches to Testing and Assessment

2 The Current Approach to Regulatory Testing and Risk Assessment

LIST OF KEY TERMS*
Active Ingredient:
The component within a pest control product to which the intended effects may be attributed. This is the ingredient that controls the pest, and it must be clearly identified on the product label.
Animal Model:
A laboratory animal used as a human surrogate in order to identify potential adverse health outcomes due to toxicant exposure.
Apical Endpoint:
An observable outcome from an animal test that is used as an indicator of toxicity — for example, growth defects, developmental issues, tumour formation, mortality, or disease progression.
Chronic Reference Dose:
The dose to which an individual could be exposed over a lifetime with no expected adverse health outcomes. The US EPA equivalent is the Chronic Population Adjusted Dose (cPAD).
Computational Toxicology:
The use of mathematical and computer models to predict adverse effects and to better understand the mechanisms by which a particular substance elicits an effect. Bioinformatics is a discipline in this field.
Epigenetics:
Changes in an organism caused by mechanisms other than changes in the DNA sequence. These changes may persist through cell division, and may even be passed to subsequent generations, but there is no change in the underlying DNA sequence of the organism.
Formulant:
A non-active ingredient that is added to a pest control product, typically to improve its properties.
Good Laboratory Practice (GLP):
As defined by the OECD, GLP is a "quality system concerned with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived, and reported" (OECD, 2004f). GLP controls are designed and enforced to ensure results are consistent, reliable, and reproducible.
Hazard:
The inherent toxicity of the chemical of interest. This is an intrinsic property of the substance.

continued on next page

*Key terms as used by the Panel throughout this report. Additional terms are listed in the Technical Glossary in Appendix A.

LIST OF KEY TERMS *(continued)*
in silico:
Performed on a computer or by computer simulation.
in vitro:
In an artificial biological environment outside of a living organism.
in vivo:
Within a living organism. For example, toxicity tests conducted using animal models.
Neurotoxicity:
The ability of a substance to cause adverse effects on the nervous system.
No Observed Adverse Effect Level (NOAEL):
An exposure level at which there is no statistically or biologically significant increase in adverse effects in the exposed population as compared to the appropriate control.
Pest:
Any injurious, noxious, or troublesome insect, fungus, bacterial organism, virus, weed, rodent, or other plant or animal.
Pest Control Product (PCP):
Any product, device, organism, substance, or thing that is manufactured, represented, distributed, or used to control, prevent, destroy, mitigate, attract, or repel a pest (Government of Canada, 2002a). All PCPs sold in Canada must have a product label that includes specific information about the active ingredients, the formulation, the intended use of the product, and the identity of the registrant. This label is a legal document that follows a standardized format.[7]
Pesticide:
The end-use pest control product. The pesticide typically contains a mixture of active ingredient and formulants.
Risk:
The likelihood that the subject will be harmed, or experience an adverse health outcome, if exposed to a particular hazard. Risk is a function of both the probability of exposure and the intrinsic hazard of the substance.

2.1 A BRIEF INTRODUCTION TO PESTICIDE TESTING AND REGULATION

The active ingredients in pesticides are one of the most stringently regulated groups used in commerce; the toxicological assessment (laboratory studies) of the active ingredient follows a regimen that is similar to that for the preclinical

7 A more comprehensive description of a pesticide label can be found at http://www.agf.gov.bc.ca/pesticides/k_2.htm

assessment for the safety of a prescription drug. These data are used by risk assessors to evaluate the ecological risks, the human health risks (including those from residues in foods), and risks for occupational exposures before they can be registered for sale or use in Canada.

In order to register a pesticide for use in Canada, an applicant (the manufacturer of the product) must submit the results of detailed scientific studies to the Pest Management Regulatory Agency (PMRA) of Health Canada as evidence of the product's safety and value (PMRA, 1999).[8] These data are used to establish "reasonable certainty that no harm to human health, future generations, or the environment will result when a product is used according to label directions" before a product can be approved for use or sale in Canada (PMRA, 2009b).

The manufacturer completes a battery of prescribed toxicity tests and exposure studies to generate data that the PMRA uses to inform an independent risk assessment (summarized in PMRA, 2009b). This risk assessment process considers not only the toxicological profile of the chemical (as determined by the toxicity test data) but also the intended patterns of use, and the efficacy of the product (PMRA, 2000). After the risk assessment is complete, the pesticide is either registered for sale and use in Canada or the registration application is rejected.[9]

This safety evaluation process has evolved into what is now known as risk assessment and risk management. This chapter outlines the risk assessment framework used to regulate pesticides and introduces the current paradigm of toxicity testing upon which regulation and risk decisions are based.[10]

2.1.1 The Importance of Regulation

The decision to use pesticides involves considering both the potential risks to human health or the environment from the pesticide itself and the issues that may occur if the pest is allowed to go unchecked.[11] Every pesticide has the potential to

8 Potential registrants generate the scientific data necessary to meet the requirements of registration. As signatories to the Organisation for Economic Co-operation and Development (OECD) Mutual Acceptance of Data (MAD) agreement (OECD, 1983), each regulatory body in an OECD country conducts an independent evaluation of a prescribed set of test data that has been produced according to GLP (PMRA, 2005). Furthermore, each country conducts its own independent review of the data and is not bound by the conclusions of the toxicity review conducted by any other country.

9 It is important to note that the registration decision describes specific crops and pest situations for which the product may be used. Depending on the outcome of the risk assessment, additional restrictions may also be imposed.

10 The Panel acknowledges that there are several categories of pesticides, including those that are biological; however, this report will focus on the evaluation of chemical pesticides.

11 Note that the Panel is specifically referring here to the decision of an individual to "use" a pesticide. It is not referring to the regulatory decision to "register" the pesticide.

harm the environment or human health to varying degrees and so too can pests that go uncontrolled; unmitigated growth of certain pests can have devastating consequences. When carefully administered, pesticides can be effective tools for the control of harmful pests before they become a threat. In fact, the primary reason for the use of pesticides in Canada and around the world is as a safeguard to control pests that otherwise might compromise food production, safety, and public health (Hillebrandt, 1960; Pretty, 2008).

Even though the costs of developing new pesticide active ingredients have increased markedly since the steady growth period of this industry in the 1970s and 1980s, new chemicals are introduced to the market each year.[12] All of these products are evaluated and assessed by individual national governments to ensure that they meet current health, environment, and safety standards.

2.1.2 Interjurisdictional Responsibilities for Pesticide Regulation in Canada

The legislation that provides the basis for environmental stewardship in Canada occurs under the *Constitution Act*.[13] In this Act, the federal government was given the authority "to make Laws for the Peace, Order and Good Government of Canada," while provinces were afforded broad jurisdiction over property and civil rights, the working environment, and waste disposal (Government of Canada, 1982).

Although the federal government is responsible for the registration of pest control products in Canada, all three levels of government (federal, provincial/territorial, and municipal) play a role in regulating their sale and use (Table 2.1). It is a complex multijurisdictional system that is affected by various acts, regulations, guidelines, directives, and bylaws across different levels of government.

Federal Regulation: The Pest Management Regulatory Agency (PMRA)

In Canada, pesticide products have been subject to regulation since 1927 through the *Agricultural Economic Poisons Act* (Dominion of Canada, 1927), which in 1939 provided the foundation for Canada's first *Pest Control Products Act* (Dominion of Canada, 1939). Early legislation was concerned with guaranteeing that products were labelled correctly and were not purposely adulterated.

12 During the period 1997–2009, a total of 109 new pesticide active ingredients were registered in the U.S. (US EPA, 2010g). In 2008–2009, 14 new pesticide active ingredients were registered in Canada (PMRA, 2009b).

13 *The Constitution Act* was originally an act of the British Parliament, referred to as the *British North American Act of 1867*. It became the *Constitution Act* when repatriated to Canada in 1982 and is formally cited as the *Canada Act 1982* (U.K.), ch 11 (Parliament of the United Kingdom, 1982).

Table 2.1

Summary of interjurisdictional responsibilities that affect the registration, sale, and use of pesticides in Canada

Level of Government	Role in Pesticide Regulation
Federal	**Health Canada** is responsible for research into acceptable levels of pesticides in prepared foods and for the conduct of biomonitoring research and studies.
	Pest Management Regulatory Agency (PMRA) is an agency that operates within Health Canada. PMRA is responsible for: • Enforcement of the *Pest Control Products Act* (PCPA); • Registration of new pesticides and re-evaluation of existing pesticides (15-year cycle); • Evaluation of effects on human health and safety, including the setting of Maximum Residue Limits (MRLs) under the *Food and Drugs Act*; • Evaluation of environmental impacts; • Evaluation of the value and efficacy; • Development of alternative strategies to minimize risk and environmental contamination; and • Post-registration oversight including compliance and enforcement.
	Agriculture and Agri-Food Canada (AAFC), in collaboration with PMRA and the provincial/territorial authorities, works with Canadian farmers (the end users of pesticides) to reduce human health and environmental risks from pesticide use. AAFC and PMRA jointly administer the Minor Use Pesticides Program.
	Canadian Food Inspection Agency (CFIA) is responsible for monitoring pesticide levels in domestic and imported foods according to MRLs set by PMRA.
Provincial and Territorial	**Provincial and territorial governments** have the authority to regulate transportation, sale, use, storage, and disposal of pesticides. Province/territory-specific regulations address: • Classification of pesticides for sale and use; • Vendor/dispenser licensing and applicator certification, training and licensing; • Grower and vendor certification; • Permits for restricted class pesticides; • Posting/notification; • Transport, storage, and disposal; and • Compliance and enforcement.
Municipal	**Municipalities**, under provincial legislation, may have the right to enact bylaws that further restrict the use and sale of certain types of pesticides (typically lawn, turf, or garden products).

Over time, incremental changes have been introduced into the regulatory landscape in response to changes in public interest and values and a growing awareness of factors that impact environmental, economic, and human health (Figure 2.1). This is reflected in the current iteration of the *Pest Control Products Act* (PCPA), which was designed to better protect human health and the environment, to make the regulatory system more transparent to the public, and to strengthen the post-registration control of pesticides (Government of Canada, 1969, 2002a).

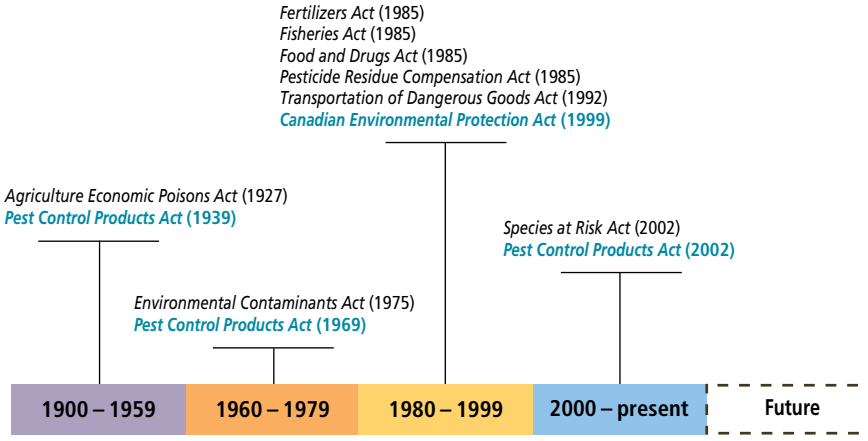

Figure 2.1
A history of federal pesticide regulation in Canada

A significant change in Canadian pesticide regulation occurred in 1995, with the establishment of the PMRA within Health Canada and the concomitant transfer of the responsibility for the regulation of pest control products from Agriculture Canada.[14; 15]

Federal Legislative Authorities:

The use of pesticides has implications for both environmental and human health. As a result, pesticides are regulated (directly or indirectly) under several Canadian laws. Table 2.2 provides an overview of the acts that either directly or indirectly impact pesticide use. The *Pest Control Products Act* (PCPA) gives the federal

14 PMRA: http://www.hc-sc.gc.ca/cps-spc/pest/index-eng.php
15 The portfolio was renamed "Agriculture and Agri-Food Canada" from "Agriculture Canada" in 1995.

government legislative authority to regulate pesticides in Canada (Government of Canada, 2002a). It requires that all pesticides that are used and sold in Canada be registered in accordance with the requirements defined and administered by the PMRA. Re-evaluations of registered products occur every 15 years although if unanticipated hazards or risks are identified, a re-evaluation can be triggered earlier (Government of Canada, 2002a). The PCPA is supplemented by the pest control products regulations. These regulations stipulate the premarket testing and registration requirements for pesticides; the conditions under which a pesticide registration may be cancelled or suspended; and the product packaging and labelling rules. Additional regulations outline the requisite fees for product evaluation and registration certificate maintenance (Government of Canada, 2006b, 2009).

Table 2.2

Federal legislative acts involving the control of pest management products

Federal Legislation of Pesticide Products	Administrative Body	Legislative Requirements
Pest Control Products Act (PCPA)	Health Canada: Pest Management Regulatory Agency	The *Pest Control Products Act* (PCPA) is the primary legislation for pesticide regulation in Canada. It requires that all pesticides are registered before they can be sold or used in Canada (Government of Canada, 2002a). The PCPA empowers PMRA to mandate that manufacturers supply sufficient toxicity data to satisfy regulators that a pesticide is efficacious and does not pose an unacceptable risk to human, plant, or animal health.
Canadian Environmental Protection Act (CEPA)	Health Canada and Environment Canada	The *Canadian Environmental Protection Act* (CEPA) gives the federal government the legislative authority for safeguarding the environment and human health from polluting substances, particularly those recognized as toxic (Government of Canada, 1999).• CEPA mandates Health Canada and Environment Canada to work in partnership in assessing potentially toxic substances, and to develop regulations for their control. CEPA provides grounds for seeking to eliminate, wherever possible, the most toxic and persistent chemicals that remain in the environment for extended periods of time and may bioaccumulate; therefore, many pesticides that are used across Canada are regulated under both CEPA and PCPA.

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• Under CEPA, "toxic" is defined in terms of persistence, bioaccumulation, and inherent toxicity.

+ The Canadian Food Inspection Agency is part of the agriculture portfolio but is separate to Agriculture and Agri-Food Canada. The president of CFIA reports directly to the Minister of Agriculture and Agri-Food.

◆ Defined as "an extirpated, endangered or threatened species, or a species of special concern."

Table 2.2 (continued)

Federal legislative acts involving the control of pest management products

Federal Legislation of Pesticide Products	Administrative Body	Legislative Requirements
Food and Drugs Act (FDA)	Health Canada	The *Food and Drugs Act* (FDA) covers foods, drugs, cosmetics, and therapeutic devices. Health Canada is responsible for establishing the standards for all food, drugs, natural health products, cosmetics, and medical devices sold in Canada. All health and safety standards under the FDA are enforced by the CFIA. PMRA establishes MRLs for pesticides during the risk assessment process. The CFIA is responsible for verifying pesticide residue levels in foods and other products at the point of sale to ensure that they do not exceed the established MRLs (Government of Canada, 1985c).
Transportation of Dangerous Goods Act	Transport Canada	The *Transportation of Dangerous Goods Act* ensures that operators receive appropriate training, and that paperwork is completed, vehicle placards are used, and safety procedures are adhered to when hazardous chemicals are transported (Government of Canada, 1992).
Fertilizers Act	Canadian Food Inspection Agency+	*The Fertilizers Act* mandates registration of all fertilizer and pesticide combinations before they can be approved for sale or use in Canada (Government of Canada, 1985a).
Fisheries Act	Fisheries and Oceans Canada and Environment Canada	The *Fisheries Act* makes it an offence to put harmful substances, including pesticides, into water frequented by fish (Government of Canada, 1985b).
Migratory Birds Convention Act	Environment Canada	*The Migratory Birds Convention Act* makes it an offence to deposit any harmful substance, including pesticides, on water or land that is frequented by migratory birds (Government of Canada, 1994a).
Species at Risk Act (SARA)	Environment Canada	The *Species at Risk Act* (SARA) is intended to protect Canadian native species of wildlife from extinction; to help in the recovery of endangered or threatened species; and to prevent other species from becoming at risk (Government of Canada, 2002b).◆ SARA prohibits the killing, harming, harassment, capture, taking, possession, collection, buying, trading, and selling of species at risk. It also prohibits the destruction or damage of a residence of a protected species. This Act however is not as explicit about pesticide applications as the US Environmental Protection Agency's *Endangered Species Act*, which ensures that any pesticides it registers will not harm endangered species (United States Government, 1973).

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Table 2.2 (continued)

Federal legislative acts involving the control of pest management products

Federal Legislation of Pesticide Products	Administrative Body	Legislative Requirements
Pesticide Residue Compensation Act	Health Canada: Pest Management Regulatory Agency	The *Pesticide Residue Compensation Act* is intended to compensate producers in the event that they incur a loss as a result of pesticide residues exceeding MRLs so long as the pesticide is used according to the label (Government of Canada, 1985d).

Provincial/Territorial and Municipal Regulation:

Provinces and territories (sometimes in collaboration with the federal government) are responsible for the training and certification of pesticide vendors and applicators and the issuing of permits for certain pesticide uses.

As discussed previously, all pesticides must be registered with the federal government before they can be sold or used in Canada; however, provinces and territories may pass laws that regulate the sale, use, storage, transportation, and disposal of registered pesticides in their own jurisdictions, provided they remain consistent and complementary to the conditions and limitations stipulated in the PCPA. A province or territory may therefore impose additional restrictions on, or prohibit the use of, a registered pesticide in its jurisdiction (reviewed in Health Canada, 2010a). Furthermore, where provided under provincial legislation, some municipalities have the right to pass bylaws that affect both the use and storage of pesticides (PMRA, 2010b). Indeed, many jurisdictions within Canada have exercised their authority in this regard, reflected by the numerous bylaws designed to restrict the sale and/or use of pesticides for cosmetic purposes (Box 2.1).[16]

Canadian Harmonization of Pesticides Regulations:

The regional differences in regulating PMRA-registered pesticides present numerous challenges for consumers and businesses alike and provide an important justification for the interjurisdictional harmonization of pesticide regulations within Canada. The Federal-Provincial-Territorial (FPT) Committee on Pest Management and Pesticides was formed to strengthen FPT relationships pertaining to pesticides;

16 The term "cosmetic pesticide" has seen common usage with respect to these bans. In this regard, "cosmetic" is deemed to be a pesticide that is used for esthetic reasons on lawns and other non-agricultural landscapes.

Box 2.1
CASE STUDY: A History of "Cosmetic" Pesticide Bans in Canada

In June 2001, the Supreme Court of Canada handed down its decision concerning the 1991 pesticide ban imposed by the town of Hudson, Quebec. The Court upheld the town's right to ban the cosmetic use of federally regulated and registered pesticides. In their unanimous decision, the justices wrote that such a law is best achieved by the level of government closest to the citizens affected. Writing on behalf of the high court, Justice Claire l'Heureux-Dubé wrote, "It is reasonable to conclude that the town bylaw's purpose is to minimize the use of allegedly harmful pesticides in order to promote the health of its inhabitants" (Supreme Court of Canada, 2001). This not only affirmed the authority of municipalities to ban pesticide use but also seemed to underscore the growing tide of health concerns related to pesticide use.

In the banning of cosmetic pesticide use, other jurisdictions are increasingly adopting a precautionary approach.[17] Since the ruling of the Supreme Court, the move to ban the cosmetic use of pesticides at the local level has gained considerable impetus. Halifax was the first large municipality in Canada to enact such a ban. It was designed to phase out the use of pesticides gradually. It took effect on 1 April 2002 but was extended to the cosmetic use of pesticides on lawns and gardens on 1 April 2003 (HRM, 2000). Over 60 Canadian municipalities and three provinces have since passed laws restricting cosmetic pesticide use. Indeed, based on 2006 census data, 77 per cent of Canadians are impacted by municipal or provincial pesticide regulation (CHO, 2010).

Facing a patchwork of sometimes contradictory municipal bylaws, the Ontario government announced in April 2008 — and in April 2009, enacted — a province-wide ban on the sale and use of urban landscape pesticides throughout Ontario, thereby superseding all other municipal bylaws in force at the time (Government of Ontario, 2008, 2009). Ontario became the second Canadian province to enact a province-wide ban on the sale and use of urban landscape pesticides, trailing Quebec by three years where the use of urban landscape pesticide has been banned since 2006.

promote information exchange; provide guidance to federal, provincial, and territorial governments in order to enhance sustainable pest management practices; and seek harmonization in programs and policies (FPT Committee, 2007).[18]

17 The precautionary approach is based on the precautionary principle which states that "Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation" (United Nations, 1992).

18 FPT Committee: http://www.hc-sc.gc.ca/cps-spc/pest/part/fpt/reference-modalites-eng.php

In June 2007, a common framework was proposed to replace the existing federal and provincial pesticide classification systems with a single, harmonized strategy for Canada (FPT Committee, 2007). The intent was to provide homeowners across Canada with consistent information and instruction on the safe use and application of potentially harmful pesticide products; however, significant differences in a number of recent provincial acts and regulations limiting the use and sale of cosmetic pesticides (Box 2.1) present a serious barrier to the implementation of this framework. Legislation regulating the cosmetic use of pesticides has been passed in Quebec (Gouvernement du Québec, 2002), Ontario (Government of Ontario, 2008, 2009), and New Brunswick (Government of New Brunswick, 2009). Legislation is pending in Nova Scotia and under consideration in British Columbia. It is likely that other provinces will also pass similar legislation (FPT Committee, 2007).

2.2 INTERNATIONAL COOPERATION ON PESTICIDES REGULATION

The frameworks that are used to regulate pesticides are designed to ensure that any human health and environmental risks posed by their use are acceptable, minimized, or essentially eliminated. The use of these frameworks by individual countries is necessary to support legitimate public policy objectives. Nonetheless, the application of different national requirements leads to significant duplication of effort for both industry and governments, which is expensive and necessitates the use of large numbers of animals to satisfy the toxicity testing requirements of each jurisdiction. In addition, different national requirements can, in some cases, create non-tariff and technical barriers to trade (OECD, 2007a).

As a result, several international organizations are involved in initiatives to minimize impediments to the international trade of chemicals while ensuring the protection of human health and the environment. Some of these organizations are responsible for the development of regulatory policies; others exist to inform policy development but are not themselves regulatory in nature. The main international organizations responsible for this work, and the programs they manage, are introduced in Table 2.3 and will be discussed in more detail throughout the report.[19]

19 It is important to note that every country retains the sovereign right to accept or reject an application. As a result, there is no obligation for each country to reach an identical conclusion to any other country with respect to the final decision to accept or reject a pesticide registration application. For example, the rate of approved application may be higher in one country because the pest pressure is different; a use may not be approved in another country because the crop is not grown there, etc.

Table 2.3
Major international efforts impacting pesticide regulation

	Function	Scope
OECD	Non-regulatory	Global
WHO/FAO	Non-regulatory	Global
Codex Alimentarius (WHO/FAO)	Regulatory	Global
EFSA	Non-regulatory	European
NAFTA	Regulatory	North American

2.2.1 The Organisation for Economic Co-operation and Development

The Organisation for Economic Co-operation and Development (OECD) is the foremost international source of chemical testing guidelines used by government, industry, and other laboratories.[20] In 1988, OECD member countries endorsed the Mutual Acceptance of Data (MAD) agreement (OECD, 1983). This agreement commits member countries to accepting test data that have been produced in another member country so long as the tests were conducted in accordance with the OECD Test Guidelines and Principles of Good Laboratory Practice (GLP) as outlined in the annex to the MAD agreement. GLP allows a reviewer to determine exactly how a test was conducted and is designed and enforced to ensure that test results are consistent, reliable, and reproducible.

The MAD agreement has had a significant impact. The number of tests that are submitted in support of a single pesticide registration is extensive with the average cost of performing these estimated at around €17 million (OECD, 2010a). Having to produce a new data set for each market would be a huge undertaking in terms of economic costs, presenting a significant barrier to trade. A recent review of the economic impact of this agreement estimates that the savings to the pesticide and chemicals industry exceeds €160 million (approximately C$225 million) per year (OECD, 2010a).[21]

To minimize duplication of data evaluation efforts and improve the efficiency of pesticide registration and re-registration, the OECD established its Pesticides Programme in 1992. This led to the formation of the Working Group on Pesticides (WGP) in 1994, which brought together representatives from government,

20 OECD: http://www.oecd.org/home
21 Assuming an exchange rate of approximately C$1.39 per €.

industry, and other groups of stakeholders. The WGP has worked to streamline the registration process (OECD, 2010b) and has realized the following advances:

- Collaborative reviews of pesticides in order to facilitate the timely sharing of assessment reports. To date, the review schedules of over 1,300 active ingredients have been posted in the public database.[22]
- The development of a standard dossier format that pesticide companies can use to submit data in support of pesticide registrations.[23]
- Harmonized review reports (monographs) that are written by a regulatory agency after its review of the submitted data set.[24]

The WGP published a vision for global harmonization of the regulatory system for agricultural pesticides for the year 2014 (OECD, 2004c). The aim is to share the data reviews of one country or region (prepared using uniform monographs) to support independent risk assessments and regulatory decisions in other regions or countries (OECD, 2009i).

2.2.2 The United Nations

The United Nations (UN) was founded in 1945 and includes 193 member states from around the world.[25] Among its objectives are the promotion of social progress, better living standards, and human rights. The UN has numerous subsidiary bodies, including both the World Health Organization (WHO) and the Food and Agriculture Organization (FAO).

Both WHO and FAO play major roles in the promotion of international efforts to minimize the risks posed by pesticide use through numerous initiatives (summarized in Table 2.4) including the Codex Alimentarius Commission, which will be discussed in more detail in the following subsection.

The Codex Alimentarius Commission of the United Nations:

While it is reasonable to assume that all consumers have the right to expect their food to be safe, of good quality, and suitable for consumption, many countries lack the institutional capacity to evaluate foods and establish science-based

22 OECD Database on Pesticide/Biocide Reviews: http://www2.oecd.org/pestdata/index.asp

23 According to the Environmental Health and Safety (EHS) Division of the OECD, the total cost of assembling a dossier in support of a pesticide registration application (not including the cost of testing) is €195,300. Industry estimates report that the use of the OECD dossier saves approximately 68 per cent of costs in developing a dossier for the same pesticide in a second country (OECD, 2010a).

24 A recent report by the OECD estimates that a comprehensive review of a full industry dossier submitted in support of a pesticide registration takes approximately 2.2 person years to complete (OECD, 2010a).

25 United Nations: http://www.un.org/en/

Table 2.4

A brief summary of WHO/FAO initiatives to pesticide regulation

Initiative	Description
Promotion of integrated pest management strategies	Integrated Pest Management (IPM) uses different techniques to control pests (EPA, 2011). In most fields, pests have natural enemies and IPM uses a pest's natural predators to protect crops. The IPM approach may not eliminate pesticide use, but it keeps it to a minimum. Pesticides are used only when it can be proven that the costs are worth it, and that there will be no harmful effects on community health and the environment. Over the past 20 years, more than 50 countries have incorporated some form of natural pest control in their domestic agricultural policy.
Facilitation of international agreements on pesticides	FAO established the *International Code of Conduct on the Distribution and Use of Pesticides* (FAO, 1985). This voluntary code, amended in 2002, helps ensure that pesticides are manufactured, packaged, transported, managed, stored, and disposed of in ways that pose the least possible risk to human health and the environment (FAO, 2005). It describes the shared responsibility of government, industry, and international organizations to regulate pesticides globally. It also encourages the implementation of integrated pest management to mitigate both human and environmental health effects by both public and private bodies (FAO, 2005). FAO also helped negotiate the Rotterdam Convention, which ensures that trade in extremely hazardous pesticides is closely monitored and restricted.
Facilitation of international food standards	The Codex Alimentarius Commission was established to protect the health of consumers, to facilitate fair trade practices in the food trade, and to promote the coordination of food standards work conducted by governments and NGOs. The Commission draws on the advice of numerous subsidiary bodies and independent committees in order to inform its standards and recommendations.
Promotion of international harmonization of regulatory standards	WHO acts as the operating agency for the International Programme on Chemical Safety (IPCS), a cooperative initiative that includes the WHO, the International Labour Organization, and the United Nations Environment Programme. The IPCS Harmonization Project (Project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals) is an effort to develop internationally accepted basic principles for risk assessment, and to provide guidance for their application in individual cases (WHO, 2011). The primary objectives of the IPCS are to establish a scientific basis for human health and environmental risk assessment and to provide technical assistance to countries in order to improve their internal capacity for sound chemical management.• WHO Human Health Risk Assessment Toolkit: Chemical Hazards (WHO, 2010) was developed through the IPCS Harmonization Project to guide the information acquisition and usage in the assessment and characterization of chemical hazards and health risks in human populations at the local or national level. It was created under the aegis of the Inter-Organisation Programme for the Sound Management of Chemicals (IOMC), a joint initiative involving a number of international groups to foster cooperation and coordination on chemical safety issues.◆ WHO Toolkit contains instructions for carrying out risk assessments, describes what information is necessary, and includes an extensive listing of international risk assessment resources for public health decision-makers. Other activities related to chemical hazards, such as risk management, risk communication, and environmental risk assessments fall outside of its parameters.

• IPCS: http://www.who.int/ipcs/about_ipcs/en/index.html

◆ The participating organizations in the IOMC are the Food and Agriculture Organization (FAO), the International Labour Organization (ILO), the United Nations Environment Programme (UNEP), the United Nations Industrial Development Organization (UNIDO), the United Nations Institute for Training and Research (UNITAR), WHO and the OECD. The World Bank and the United Nations Development Programme (UNDP) are observers.

safety standards. The Codex Alimentarius Commission (CAC) was established as a joint initiative between WHO and FAO in 1961 in order to provide a single international reference point for developments associated with food standards (Codex Secretariat FAO, 2006). The CAC produces the Codex Alimentarius, which is a collection of science-based standards, codes of practice, guidelines, and other recommendations related to a given commodity. The Codex Alimentarius established Maximum Residue Limits (MRLs) for pesticides in food.

The standards, guidelines, and other recommendations promoted by the CAC are developed through the work of its subsidiary bodies, which in turn are supported by the work of expert advisory groups such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR).

Joint FAO/WHO Meeting on Pesticide Residues:
JMPR consists of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group, and it is administered jointly by FAO and WHO.[26] JMPR is an independent, international group of scientific experts who, since 1963, has been responsible for the review of pesticide-related data in order to determine if pesticide residue values truly represent a safe dietary intake level for the various populations of the world.[27]

JMPR evaluations are used to ascertain the safety of the proposed MRLs (i.e., the levels of pesticide residue that are tolerated on food commodities in international trade). Thus, JMPR serves as a scientific advisory body to the CAC. Advice to the CAC on pesticides is provided through the Codex Committee on Pesticide Residues (CCPR).[28] For many developing countries this is the primary safeguard for ensuring the safe agricultural use of pesticides. The JMPR is the only international body performing these critical functions, and its work is recognized by the World Trade Organization as the standard.

Joint FAO/WHO Expert Committee on Food Additives:
JECFA is an international expert scientific committee, established in 1956 and also administered jointly by WHO and FAO.[29] JECFA evaluates the safety of food

26 JMPR: http://www.codexalimentarius.net/web/jmpr.jsp
27 This includes a review of all toxicology, metabolism, environmental fate, patterns of use, residues, and analytical data related to pesticides.
28 The Codex Committee on Pesticide Residues identifies substances that require priority evaluation. These substances are then referred to the JMPR for recommendations on MRLs and sampling methods.
29 JECFA: http://www.codexalimentarius.net/web/jecfa.jsp

additives, including contaminants, naturally occurring toxicants, and residues of veterinary drugs in foods.

2.2.3 The European Food Safety Authority

The European Food Safety Authority (EFSA) was established in 2002 to provide independent scientific advice on issues pertaining to food and feed safety (EFSA, 2009b; EU, 2002).[30] EFSA's work, which includes data collection, data analysis, and the identification of emerging risks, is divided into two main areas of focus: risk assessment and risk communications. The opinions and assessments conducted by EFSA are used to inform legislation and regulatory measures by the European Commission, European Parliament, and the European Council.

2.2.4 The North American Free Trade Agreement Technical Working Group on Pesticides

The North American Free Trade Agreement (NAFTA) Technical Working Group on Pesticides (NAFTA-TWG) was formed in 1996 to align the individual pesticides registration systems of Canada, the United States, and Mexico (NAFTA-TWG, 2009a).

The NAFTA-TWG has three main strategic objectives relevant to pesticide regulation and risk assessment:

- To provide pesticide users with equal access to pest management tools and to provide access to lower-risk alternative products where applicable.
- To maximize the use of each nation's re-evaluation program and review of older pesticides to facilitate the removal of unsafe products from the market.
- To provide a harmonized set of regulations across Canada, the United States, and Mexico in order to reduce the costs to government and industry of regulating and reviewing the same data sets.

At a recent meeting, the NAFTA-TWG received approval from the Executive Board to develop a new project on 21st century toxicology. The objective of this project is to "supplement, replace and reduce traditional animal toxicity testing methods and risk assessment by using a variety of tools and approaches in combination" (NAFTA-TWG, 2009b).

2.2.5 The Role of Canada in International Cooperation of Pesticide Regulations

Canada is an active participant in the international cooperative initiatives described in the previous sections. This reduces duplication of efforts with respect to data

30 EFSA: http://www.efsa.europa.eu/

collection and facilitates the expeditious review of newer, reduced-risk products (PMRA, 2009b). As of 31 March 2009, 35 active ingredients had been registered in cooperation with other international regulatory bodies (with five joint reviews involving coordination with the U.S. and Mexico or other international bodies) (PMRA, 2009b).

Members of PMRA are invited to serve as delegates on the OECD Working Group on Pesticides and on the JMPR and JECFA because of their expertise. They normally do so as individuals, not as representatives of the PMRA. In these roles, individuals from Canada are able to contribute to discussions about the coordination of domestic and international policies for assessment of pesticide risk.

2.3 THE ASSESSMENT AND MANAGEMENT OF PESTICIDE RISKS

Regulation of pesticides is based on risk assessment and risk management. The purpose of risk assessment is to answer the question: "What is the risk that exposure to a particular hazard (e.g., a pesticide) will result in harm?" Risk management then seeks to mitigate this risk and evaluate the impacts of regulatory measures on the risk (NRC, 1983).[31]

(Council of Canadian Academies)

Figure 2.2
Risk is a function of hazard, exposure, and susceptibility

31 In this regard, exposure captures the dose to which the individual is exposed.

Risk is a function of both the inherent toxicity of the chemical of interest (i.e., its hazard) and the probability of sufficient exposure to elicit an adverse health effect in a susceptible individual (Figure 2.2). Hazard is therefore an intrinsic property of the chemical of interest; susceptibility is inherent to the affected organism; and exposure is a result of the environment into which the chemical is released (Health Canada, 2002).

The risk assessment process was initially codified in 1983 by the publication of the "Red Book"[32] (NRC, 1983). The principles and process described in this paradigm have since been integrated into the risk assessment practices used in many parts of the world; they still provide the fundamental structure for pesticide risk assessment today (Box 2.2).

Box 2.2
Core Principles of the Red Book Framework

The traditional risk assessment framework, as described in the original Red Book, includes four distinct steps that are described below:

1. Hazard Identification: Determines whether exposure to a particular chemical agent has the potential to produce adverse health effects in humans. This information is based on data obtained from testing chemical effects *in vivo* (typically in a rodent model).[33]
2. Dose-response Assessment: Determines the relationship between the adverse effects and the dose of the chemical agent; takes into account the frequency of exposure and factors such as gender and age; extrapolates from data obtained from animal-based *in vivo* toxicity tests.
3. Exposure Assessment: Measures or estimates the frequency or duration of exposures by humans to the potential chemical agent; takes into account routes, types (occupational or residential) and frequency of exposure.

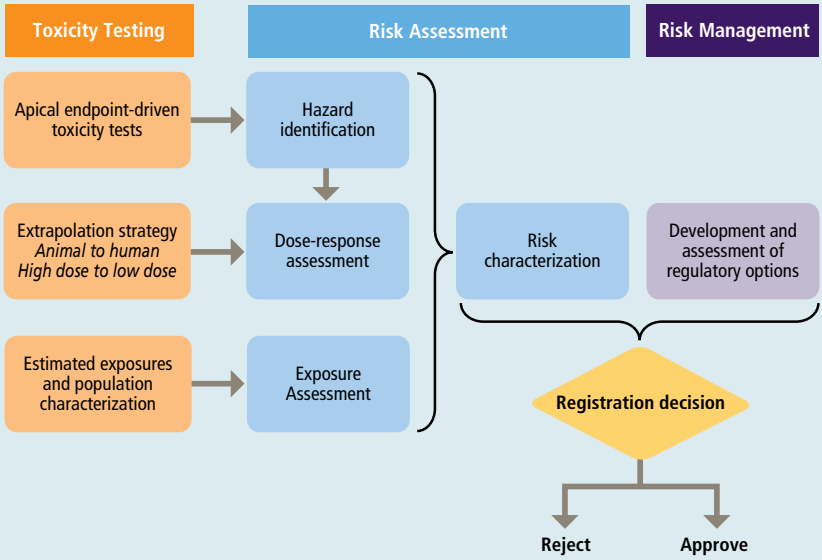
continued on next page

32 This book, *Risk Assessment in the Federal Government: Managing the Process*, is more commonly referred to as the "Red Book."

33 Hazard is also considered in the context of an environmental risk assessment, where it would be assessed using data from a wider range of *in vivo* species.

Box 2.2 *(continued)*

4. Risk Characterization: Estimates the risk of negative health effects under the conditions determined in the exposure assessment; uses the information from the dose-response assessment and the exposure assessment.



(Adapted and reproduced from Risk Assessment in the Federal Government: Managing the Process, 1983 with permission from the National Academy of Sciences, Courtesy of the National Academies Press, Washington, DC)

The risk assessment framework of the National Research Council of the United States

The hazard, dose-response, and exposure assessments comprise the detailed technical analyses; characterization of these factors is used to select the appropriate data upon which conclusions are based. The 1983 framework was updated in 1994 in a report that emphasized the need to transparently describe the scientific and policy basis for risk assessments and to characterize the strengths, limitations, uncertainty, and variability associated with health risk estimates (NRC, 1994).

The 1994 report also recommended that risk assessment should be an iterative process that identifies key information needs and allows for improvement in the risk assessment as applicable to the decision-making level (NRC, 1994).

The 1983 risk assessment framework made a clear delineation between risk assessment and risk management; this was amended in a subsequent revision in 2009, which suggested the utility of a risk assessment would be increased by dialogue between risk assessors and risk managers early in the planning process. Furthermore, the inclusion of other stakeholders in that dialogue would ensure that the technical analyses within the risk assessment would align more closely with the risk management options and questions to be answered (NRC, 2009).

2.3.1 Risk Assessment of Pesticides in Canada

In order for a pesticide to be registered for use in Canada, the PMRA must be satisfied that there is "reasonable certainty that no harm to human health, future generations, or the environment will result when a product is used according to label directions" and that the product is efficacious (PMRA, 2000, 2009b).

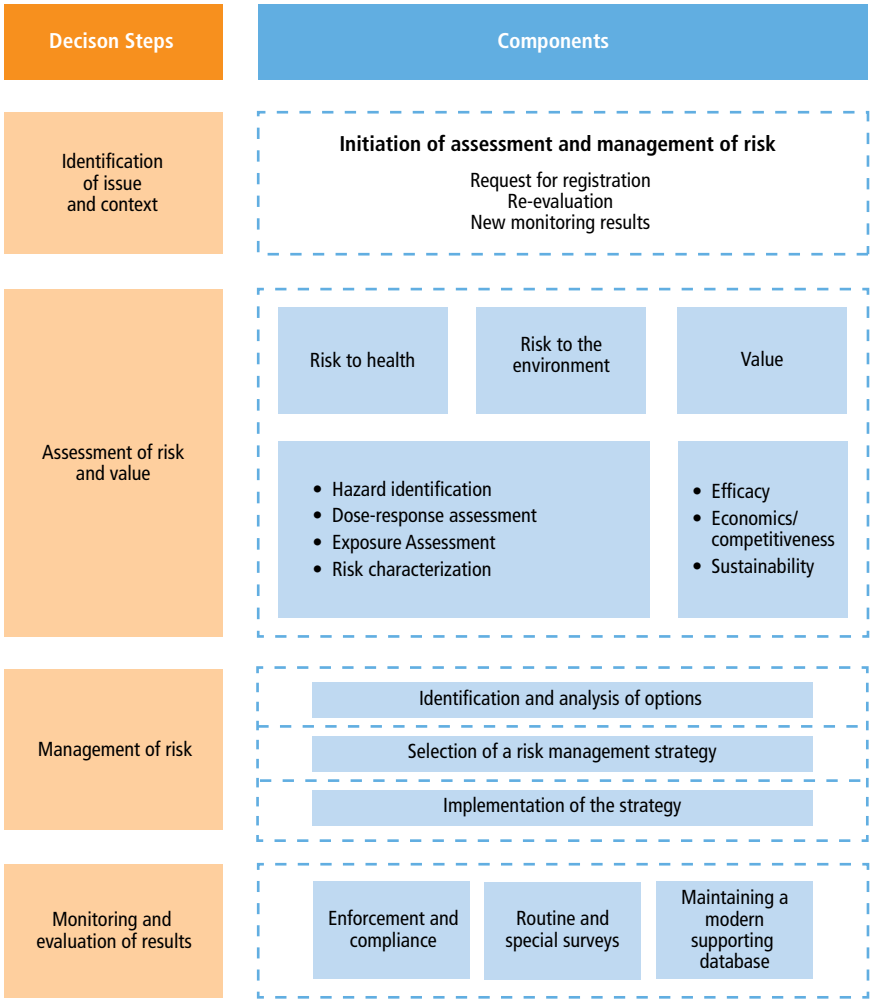
The decision framework used by PMRA is shown in Figure 2.3. This framework incorporates the principles described in the Red Book (Box 2.2) into a more extensive decision-making framework (PMRA, 2000). It is consistent with international best practices (IPCS, 1999, 2009b) and includes four decision steps, of which the core principles of risk assessment discussed above constitute an integral but non-exhaustive component:

- identification of issue and context;
- assessment of risk and value;
- management of risk; and
- monitoring and evaluation of results.

Individuals may be exposed to several different kinds of pesticides over their lifetimes (cumulative) and through different exposure routes (aggregate). This must also be taken into account in risk assessments (Box 2.3).[34]

PMRA conducts risk assessments using a prescribed data set produced from a suite of primarily *in vivo* tests that are performed in order to compile a thorough set of toxicity data. These tests will be discussed in the following section.

34 For more information refer to PMRA, 2003.



(Reproduced with permission from Health Canada)*

Figure 2.3

The decision framework of the Pest Management Regulatory Agency in Canada

*Science Policy Notice SPN2000-01 Technical Paper: A Decision Framework for Risk Assessment and Risk Management in the PMRA, Health Canada, 2000. Reproduced with the permission of the Minister of Health, 2011.

Box 2.3
Pesticide Risk Assessments are Scenario-Based

Risk assessments consider multiple factors in order to evaluate a pesticide: the intended use; the potential routes of exposure; and the groups of individuals that are likely to be exposed. In particular, occupational and residential exposure scenarios necessitate specific information and methods for estimating risk from various routes at varying frequencies and durations of exposure. Several risk assessments are conducted to address the potential exposure scenarios based on the product's intended purpose and pattern of use.[35]

In residential applications, as in all other applications, multiple routes of exposure may need to be considered. These include residue uptakes through the skin, by inhalation, and incidental oral ingestion such as hand-to-mouth behaviour (especially by children). Toxic effects can differ by the route of exposure, and there can be portal-of-entry effects at the site of contact. Studies based on dermal exposure or inhalation are not often available so route-to-route extrapolations may be necessary.

Like the residential exposure scenarios, the assessment of occupational risk also requires consideration of different routes and times of exposure. In this case, these are determined by the nature of the work situation and practice. Inhalation and dermal are the main routes of exposure, and the durations of exposure range can from short to long term. The extent of occupational exposure will depend on a number of factors including application rate, method and frequency of application, product formulation, amount of pesticide handled, mixing/loading operations, type of application equipment, and re-entry time in treated areas. All of these factors must be addressed during the risk assessment process.

When evaluating agricultural pesticides, a dietary risk assessment is conducted to address exposures that might occur from eating foods containing pesticide residue and from drinking water where residues may have migrated into surface water and groundwater reservoirs. The risk assessment is used to support the establishment of a Maximum Residue Limit (MRL) as mandated by the *Food and Drugs Act* (Government of Canada, 1985c). The MRL, expressed in parts per million (ppm), is the maximum amount of pesticide residue legally permitted in or on a food commodity (see Table 2.2).

35 A summary of the human health risk assessment process for pesticides may be found at: http://www.ppp.purdue.edu/Pubs/PPP-52.pdf

2.4 THE EXISTING TOXICITY TESTING SYSTEM

The modern science of toxicology has four main themes: descriptive, mechanistic, predictive, and regulatory (Box 2.4). Each of these thematic areas integrates data and expertise from a wide variety of scientific disciplines. Together, these areas form the foundation upon which the field of regulatory risk assessment was developed.

Box 2.4
Thematic Toxicology

Descriptive toxicology is primarily concerned with the development and execution of toxicity tests that produce data to inform safety evaluations and regulatory decision-making processes.

Mechanistic toxicology aims to understand how different agents exert their effects on biological systems.

Predictive toxicology seeks to use existing information from known toxicants to infer the toxicological profile of related chemicals and predict pathogenesis.

Regulatory toxicology integrates the information and data derived from descriptive and mechanistic toxicology with exposure information to determine whether the agent in question poses an unacceptable risk.

Toxicity testing is conducted in order to identify potential adverse effects in biological systems after exposure to a given chemical. The data generated in these studies are used to inform both the hazard identification and dose-response assessment of the risk assessment process discussed in Section 2.3. These data are used to characterize the potential types of hazards by biological affects (e.g., genotoxicity, neurotoxicity, developmental toxicity, and reproductive toxicity) that may be perturbed by the active ingredient. They may also be used to characterize the exposure conditions (by route and duration) under which these hazards may arise (Box 2.3).

In vivo studies have been critical to the evolution of the science of toxicology; the generation of toxicological data in animal models remains one of the cornerstones of experimental toxicology today. The use of animals in regulatory toxicology can be traced to several key events that took place in the early- and mid-twentieth century. Tests were developed in order to demonstrate the safety and efficacy of the products, often in response to a major safety problem or issue. These tests

were based on observable outcomes and, over the years, have become an integral component of the regulatory regimes that depend on them.

In vivo Toxicity Testing to Inform Risk Assessment:

PMRA is responsible for determining which tests are required, conditionally required, or not required (see Appendix B for full details) for the registration of a pesticide in Canada.[36] These data requirements are harmonized, to a large extent, with those of the United States (US EPA) and other OECD countries (PMRA, 2005).[37; 38]

Toxicity testing is broken down into several broad categories based on test duration and objectives (PMRA, 2002, 2005) (Table 2.5). In this approach, acute tests are often the first tests performed, and are used to inform dose selection and exposure duration.

The toxicity data are used to determine a No Observed Adverse Effect Level (NOAEL), which is generally the highest dose that does not cause any statistically significant adverse effects relative to controls.[39] The lowest dose level that results in a statistically significant adverse response (relative to controls) is called the Lowest Observed Adverse Effect Level (LOAEL). Alternatively, a Benchmark Dose (BMD) may be derived. A BMD is the dose projected (from a fitted mathematical model) to cause a pre-specified level of change from the control in an exposure response (EFSA, 2009; Crump, 1984; US EPA, 2002b). BMDs typically serve as the points of departure to assess the potential risks posed by the various exposure scenarios.

The BMD is then used to calculate a number of health-based and regulatory standards, including a Maximum Residue Limit (MRL), an Acceptable Daily Intake (ADI), an Acute Reference Dose (ARfD), and a chronic ADI or Reference Dose (RfD).[40] A risk is considered tolerable if the anticipated maximum one-day and

36 Note that the PMRA may modify its data requirements on a case-by-case basis in order to obtain the actual data needed to fully elaborate the characteristics or effects of specific products under review (PMRA, 2005).

37 PMRA has adopted the internationally recognized test guidelines published by the US EPA (US EPA, 2010j) and the OECD (OECD, 2009c).

38 A list of all 34 OECD member countries may be found on the OECD website: http://www.oecd.org

39 Adverse effect is defined as changes in the morphology, physiology, growth, development, reproduction, or life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (IPCS, 2004).

40 Acute refers to the dose to which an individual could be exposed in a day with no expected adverse health outcomes. The US EPA equivalent is the Acute Population Adjusted Dose (aPAD). Chronic refers to the dose to which an individual could be exposed over a lifetime with no expected adverse health outcomes. The US EPA equivalent is the Chronic Population Adjusted Dose (cPAD).

Table 2.5

A summary of the nine categories of toxicity studies used and the type of data they provide

Category	Description	Type of Data Provided
Acute toxicity studies	• Often the initial studies performed to evaluate the adverse effects of short-term or single-dose exposure to a compound via different routes of exposure (i.e., dermal, oral, or other routes) • May serve as a basis for hazard categorization or labelling, or to designate those pesticides that are applied only by certified applicators • Provide a measure of potential for lethality • Do not satisfactorily define non-lethal acute effects	• Information on health hazards likely to arise from a single or short-term exposure to the test substance including behavioural and clinical abnormalities, gross lesions, body weight changes, effects on mortality • Reversibility of observed abnormalities • Relative acute toxicities from different routes of exposure • Capacity to produce eye and skin irritation and dermal sensitization • Appropriate dose levels for subsequent toxicity studies
Short-term studies	• Evaluate the adverse effects of repeated exposure over a duration of up to 10 per cent of the animal model's lifespan • Also known as subchronic studies	• Toxic potential resulting from daily, repeat exposure • Cumulative or delayed toxicity • Variability in species sensitivity • Organ or system vulnerabilities • Used to inform dose selection for long-term chronic toxicity and carcinogenicity studies
Long-term studies	• Also known as chronic studies • Evaluate cumulative adverse effects of repeated, life-long exposure, systemic toxicity, carcinogenic potential, and dose-response characteristics • Typically conducted over at least 90 per cent of the anticipated lifespan of the test animals when conducted in rodents (substantially less when conducted in dogs) • Although chronic toxicity and carcinogenicity are often evaluated in the same study, they fulfil two different objectives and are evaluated independently	• Chronic systemic toxicity • Carcinogenic potential • Dose-response relationships and reversibility or persistence of adverse effects • Cumulative toxicity • Variability in species sensitivity
Reproduction studies	• Evaluates the potential of the chemical to affect both male and female reproductive performance such as gonadal function, mating behaviour, and conception, development of the conceptus, and parturition • Offspring of test animals are also observed to identify effects on survival, viability, development, and behaviour	• Capacity to influence reproductive performance, function • Potential adverse effects on survival, viability, development, and behaviour • Effects potentially associated with altered hormone levels • Susceptibility and sensitivity in offspring

continued on next page

Table 2.5 *(continued)*

A summary of the nine categories of toxicity studies used and the type of data they provide

Category	Description	Type of Data Provided
Developmental toxicity studies	• Test the potential of a chemical to induce structural changes, growth retardation, functional deficits, and death in the developing embryo/fetus • Also known as teratogenicity studies (measures of induced malformations)	• Critical periods of susceptibility and endpoints in developing embryo/fetus • Maternal effects
Genotoxicity studies	• Evaluate the capacity of the tested chemical to induce DNA damage and chromosomal changes • Used to infer potential for damage in mammals • Use combination of *in vitro* and *in vivo* assays	• Capacity to induce gene mutations and, chromosomal changes and reduce competency of DNA repair mechanisms
Toxicokinetic studies	• Provide information pertaining to the absorption, distribution, metabolism, and excretion (ADME) of the chemical in question • These data are also used to inform animal-to-human extrapolation decisions in the subsequent risk assessment	• Absorption, distribution, metabolism, and excretion (ADME) • Identification of toxic effects (or lack thereof) • Absorption via different routes of exposure • Used to inform dose selection and routes of administration in long-term studies
Neurotoxicity studies	• Determine whether exposure has the potential to produce adverse changes to the structure or functional integrity of the nervous system or developing nervous system • Assessed on the basis of behaviour, neurophysiology, neurochemistry, and neuropathology	• Capacity to affect the structure or function of the central and peripheral nervous systems • Sensitivity or susceptibility of the developing nervous system • Both acute and repeated dose effects can be evaluated
Immunotoxicity studies	• Evaluate the potential to elicit adverse effects on the structure or function of the immune system or other systems as a result of immune system dysfunction • Data often incorporated into the standard toxicity protocols for acute, short-, and long-term exposure studies	• Capacity to reduce/antagonize immune system response

lifetime exposures are below the calculated ARfD and chronic ADI respectively (PMRA, 2008d). For non-dietary risks (e.g., workplace or residential exposures), as well as aggregate risks from all sources of exposure, the ratio of the NOAEL to the estimated exposure is calculated in order to generate a margin of exposure (MoE) (PMRA, 2008d).

2.5 CRITIQUE OF THE CURRENT TOXICITY TESTING SYSTEM

The current regulatory paradigm relies extensively on data derived from the observation of apical endpoints from animal studies. The suite of standardized protocols for the pre-market testing of pesticides was designed to minimize variance and to provide a robust and comprehensive data set upon which to base subsequent regulatory decisions.

Although these tests have served the needs of risk assessors for several decades, many have not changed appreciably since their inception over 30 years ago. As science has evolved in recent decades, our understanding of physiology has increased immeasurably; however, these advances have not been reflected in changes to the battery of toxicity tests that are required for regulatory decision-making (reviewed in Seidle & Stephens, 2009). Many of the standardized tests that are used in the existing toxicity testing battery predate the so-called omics era and are not designed to generate (or incorporate) data pertaining to molecular mechanisms and signalling pathways.[41] Weaknesses of the existing regulatory paradigm and associated testing strategies are objectively critiqued in the following subsections.[42]

2.5.1 Predictivity and Relevance to Humans

The selection of an animal model that is practical to study yet predictive of the human response is a challenge that is inherent to any *in vivo* testing regime. Test species for many early studies were selected for their convenience and familiarity; however, as our understanding of conserved metabolic pathways across species has increased, so has the need to select models based on physiological appropriateness.

41 It is important to note that the Panel believes that decisions should be made on the basis of the best available science. Neither the age of a test itself, nor the nature of the model it uses, is problematic *per se*.

42 Note that, in contemplating future approaches to toxicity testing and risk assessment of pesticides, the Panel focused on the best available current and emerging scientific methods to accomplish this task. Although a reduction in the use of experimental animals in toxicity testing was not an *a priori* objective, it is possible that animal use may be significantly reduced in the future, both as a consequence of the trend towards greater use of *in vitro* and *in silico* test methods, and the more efficient use of animals within a tiered testing construct. At a macro level, the use of (non-animal) alternative tests may result in a more focused and efficient use of the finite toxicity testing resources available to address the universe of chemicals to which humans are potentially exposed.

Ideally, animal tests should be conducted in the species that most closely mimics the pharmacodynamic properties of the human; however, in reality the information needed to determine this is rarely available.

Over the years there has been increasing concern that certain responses observed in laboratory animals may not be predictive of the human outcome and that other effects may be missed (Olson *et al.*, 2000). Indeed, the information derived from the existing battery of toxicity tests is highly specific in nature and does not easily facilitate extrapolation of findings to other species, life stages, and susceptible populations. This has necessitated the development of policy-based adjustment factors to account for uncertainties in extrapolating test outcomes from animal studies to those relevant to human health (Box 2.5). The use of such factors,

Box 2.5
Addressing Intraspecies Variability and Interspecies Extrapolation

Regulatory agencies almost universally use uncertainty factors to address uncertainties in the scientific data upon which risk-based decisions must be made.[43] Uncertainty factors accommodate variability arising from interspecies extrapolation (animal to human), intraspecies differences (human to human), LOAEL to NOAEL extrapolation (used when no NOAEL value is available), extrapolation across dosage, and database deficiencies (PMRA, 2008d).

At a minimum, a reference dose usually incorporates two 10-fold uncertainty factors. As a result, the calculated reference dose for humans is typically at least 100-fold lower than the dose that was observed to produce no adverse effect in animal studies. The *Pest Control Products Act* also necessitates the inclusion of an additional default 10-fold factor for the protection of pregnant women, infants, and children, unless there are compelling data to support the use of a different factor (PMRA, 2008d).

Under the IPCS, an approach was developed to transparently incorporate data in order to permit science-based extrapolations (IPCS, 2005). To date, these chemical-specific adjustment factors have not seen considerable use in a regulatory context due to the lack of pharmacokinetic and mode of action information. The availability of such information will enable the use of more appropriate interspecies and human variability extrapolation factors.

43 The terms "uncertainty factor" and "safety factor" have often been used interchangeably in the past. In Canada, PMRA used "safety factor" to account for concerns related to age sensitivity or severity of endpoint. Because the adoption of the PCPA is intended to address these concerns, the term safety factor has now been abandoned (PMRA, 2008d).

although critical to the success of the current paradigm in protecting human health, is a tacit admission that the current approach to testing does not have the precision desired in toxicological assessment. It is this added precision, via mechanism-directed integrated approaches, that IATA might be able to provide.

The core and much-debated assumption at the heart of the current regulatory toxicology paradigm remains the same: High-dose testing of a relatively small group of animals produces statistically robust data that are used to predict the effects of low-dose exposures. Often the administered dose is several orders of magnitude higher than the predicted environmental exposure levels. Inferring relevance of any resultant adverse outcomes is complicated by the uncertain nature of the dose-response relationship; indeed, the utility of using high doses to predict outcomes that may result from low-dose exposures has long been questioned. High doses of a chemical may trigger responses in metabolic pathways that would not be affected at lower levels of exposure. Conversely, effects that might manifest at low dose levels would be missed because subtle interactions would remain undetected (reviewed in NRC, 2007). This presumably results in potential false positives and false negatives that are undetectable under the current approach.

Furthermore, as discussed earlier, toxic responses may differ depending on the route of exposure and path of entry of the toxicant into the body. Although some dermal or inhalation data may be available, the standardized suite of animal-based toxicity tests almost exclusively considers exposure via the oral route, which necessitates route-to-route extrapolation. Although the risk assessment process endeavours to address this by taking a scenario-based perspective (Box 2.3), it is limited by a lack of primary data on exposure via other routes relevant to humans. Incorporation of pharmacokinetic studies that would evaluate the absorption, distribution, metabolism, and excretion (ADME) of substances would greatly enhance robust route-to-route extrapolation (Barton *et al.*, 2006).

2.5.2 Inability to Evaluate Chemical Mixtures

As discussed earlier, a pesticide product contains multiple ingredients (i.e., the formulants and active ingredient or ingredients); however, the regulatory decision is based primarily on toxicity data pertaining to the active ingredient tested as an isolated chemical species, with limited (acute) toxicity testing on the final formulation proposed for registration. The lack of chronic toxicity data on pesticide formulations speaks to a much larger issue in toxicity testing, namely, the evaluation of chemical mixtures.

In reality, individuals experience combined exposures to multiple chemicals; the resultant toxicity from combined exposures may be quite different from that

exhibited following exposure to any one chemical in isolation. Depending on the mode of action (MoA) of the chemicals, their route(s) of exposure, and their target tissues, they may behave synergistically (i.e., exhibit increased toxicity) or antagonistically at a given dose; however, the existence of reliable data for environmentally relevant levels of exposure are limited (reviewed in Cox & Surgan, 2006).

Although there is no internationally agreed-upon approach to address combined exposures to multiple chemicals, several jurisdictions — including Canada, the U.S., and the EU — have implemented measures to address the risk from combined exposures to multiple chemicals (European Union, 2005; Government of Canada, 2002a; United States Government, 1996a). Furthermore, the IPCS released a workshop report (2009a) outlining a tiered approach to assessing exposure to multiple chemicals. Integral to this approach would be consideration of the MoA of the individual chemicals. A final, peer-reviewed framework is currently under development (IPCS, 2009a). (See Box 2.6 for clarification on the language used to describe mixtures, aggregate exposure, and cumulative risk.)

Box 2.6
An Aside on Chemical Mixtures, Aggregate Exposure, and Cumulative Risk

Historically, risk assessment has considered the risk posed due to exposure to a single agent; however, humans routinely experience simultaneous exposure to numerous stressors via multiple routes of exposure (e.g., inhalation, ingestion, dermal contact) over short or prolonged periods. This presents a significant challenge because, even for a defined formulation, the different environmental behaviours of the formulation will result in most individuals being exposed to a poorly defined mixture.

A chemical mixture describes the combination of substances to which an individual is exposed. In the case of a single product (e.g., a pest control product), this mixture is known and defined. In the case of environmental exposures, the mixture is variable, constantly changing, and essentially indefinable.

Aggregate exposure describes the combined exposure to a given chemical by multiple pathways (also described as "single chemical, all routes"). Cumulative risk describes the combined risks posed by aggregate exposures to multiple chemicals or stressors that share a common mechanism of toxicity (also described as "multiple chemicals, multiple routes").

Although most of the data requirements and regulatory activities pertaining to pesticide registration focus on the active ingredients, pesticide products also contain formulants. Formulants (also referred to as "co-formulants" or "inert ingredients") are part of the end-use product formulation. They are the substances (or a group of similar substances) that are intentionally included to increase the effectiveness of the product.[44; 45] Typically, multiple formulants are in a pesticide product; they may perform a wide array of functions such as solvents, carriers, emulsifiers, thickeners, and pH control agents. Formulants vary greatly in their physical and chemical characteristics, and their chemical structures are often quite different from that of pesticide active ingredients. These substances comprise a large inventory of chemicals that are used in pesticide formulations. For example, in the U.S. over 4,000 substances are identified as "inert ingredients" that could be used in pesticide products (US EPA, 2009n). The result is a substantial disparity in the data requirements of the ingredients contained within the final pesticide product. On the one hand, the active ingredient has been subject to an extensive battery of toxicity studies; on the other, for the majority of formulants there are almost no toxicity data. A list of all formulants currently used in registered Canadian pest control products is publicly available (PMRA, 2007); however, a pesticide formulation is protected as a trade secret, and only formulants that are known or suspected toxicants must be disclosed on the product label (PMRA, 2006b).[46]

Furthermore, although the toxicological properties of the active ingredient itself are studied extensively, toxicity data on the properties of its breakdown products (both metabolic and environmental) are often quite limited. The existing approval and testing scheme is not useful for analyzing these degradates; however, retrospective analyses have suggested that they may pose a greater hazard than the original pesticide active ingredient (reviewed in Andreu & Pico, 2004).

It is widely accepted that the existing approach to toxicity testing cannot address the health and environmental impact of exposure to chemical mixtures (reviewed in Lydy *et al.*, 2004) and the current *in vivo* approach cannot adequately remedy this situation. The number of possible chemical combinations to which a population may be exposed is innumerable; to test all of these using the current system would be impossible from a practical perspective.

44 Under new EU regulations, substances or preparations that are used, or intended for use, in a plant protection product or adjuvant, but are neither active substances nor safeners or synergists, are referred to as "co-formulants" (European Union, 2009c).

45 Formulants are termed "inert ingredients" in the U.S. legislation, but this is scientifically inaccurate (Stephenson & Soloman, 2007); formulants is the preferred and accepted nomenclature in Canada.

46 These are formulants that have been categorized as of significant concern with respect to potential adverse effects on health and the environment (List 1) or potentially toxic based on structural similarity to List 1 formulants or on data suggestive of toxicity (List 2) (PMRA, 2010a).

2.5.3 Limited Coverage of the Universe of Environmental Chemicals

As discussed earlier, the data-rich and data-poor nature of pesticide mixtures is a metaphor for the dichotomy that exists for most industrial chemicals. The active ingredients in pesticide products are one of the most data-rich groups of regulated chemicals on the market today. In sharp contrast, little or no toxicity data exist for many other chemicals (including some non-active ingredients found in pesticide formulations), metabolites, or environmental degradates. Recent estimates suggest that toxicity data are lacking for 87 per cent of chemicals on the market (reviewed in Hartung, 2009). Regulatory agencies are taking actions to address these issues (see Chapter 4); however, it is widely accepted that the existing *in vivo* toxicity testing approach cannot fulfill demands for increased data (reviewed in Hartung, 2009; Hartung & Rovida, 2009; Schoeters, 2010).[47]

2.5.4 The Challenge of Including Epidemiological Data

Epidemiological data are potentially very important sources of knowledge that are specifically relevant to the human experience. The current registration system for pesticides in Canada, and internationally, is largely pre-market in context; therefore, epidemiological considerations have not contributed substantially to the registration process. Furthermore, although the 15-year re-registration cycle represents an important opportunity to harness real life, field-relevant, human experience, post-market surveillance programs have not played a significant role in the re-registration process.

Traditional epidemiological studies often rely on imprecise exposure measurements in a heterogeneous population, which makes it virtually impossible to closely monitor any large population group for environmental exposure over even short periods. As a result, exposure histories are reconstructed retrospectively, whether in cohort or case-control studies, and often provide qualitative rankings rather than quantitative values. Furthermore, because many adverse health outcomes have long latency periods, in most cases it would be difficult to establish a causal relationship between exposure and adverse health outcome on a population level. Consequently, epidemiological studies in humans and toxicity studies in animals can present conflicting outcomes.

For example, the apparent differences in results obtained for the pesticide 2,4-dichlorophenoxyacetic acid (2,4-D) between *in vivo* laboratory studies and human population studies might be due to the different types of exposures evaluated.

47 For example, new EU legislation will require testing data for 30,000–101,000 substances. This range reflects the official estimates of the EU prior to the pre-registration phase of REACH, and the estimates by toxicologists following conclusion of this phase. The EU had anticipated the pre-registration of approximately 29,000 substances from 27,000 companies (Pederson *et al.*, 2003); however, the actual number was 143,835 substances from 65,000 companies (ECHA, 2010).

The animal studies used pure 2,4-D, whereas the epidemiological studies evaluated complex environmental exposures to multiple chemicals. The regulatory evaluations of governments in Canada, the U.S. and the EU have concluded that — when used properly and in accordance with label directions — 2,4-D poses little or no risk to users or bystanders, including children (European Commission, 2001; PMRA, 2008b, 2008c; US EPA, 2007a). This contrasts with epidemiological studies that have found positive associations between the residential use of 2,4-D (as a constituent of poorly characterized, mixed pesticide exposures) and cancer risks in humans and particularly children (Infante-Rivard & Weichenthal, 2007; OCFP, 2004; Sears *et al.*, 2006).

Whereas epidemiological studies on the effect of pesticides in childhood cancer are consistently positive and those on workers exposed to specific pesticides, such as 2,4-D are less consistent (Aylward, Morgan *et al.*, 2010; Infante-Rivard *et al.*, 1999; Infante-Rivard & Weichenthal, 2007; Zahm & Ward, 1998), the following point is important to underscore. In non-occupational studies (in particular those among children), the exposure most often pertains to a category of pesticides or to a mixture of pesticides rather than to any specific one due to the outstanding difficulties of retrospectively measuring exposures over a long period of time in free-living populations. On the other hand, *in vivo* animal laboratory studies can study a specific agent. Because it is unlikely that epidemiological studies in free-living populations will have access to specific pesticides exposure data anytime soon, but their results in humans often conflict with those of *in vivo* animal study results, it is important to weigh the epidemiological data and their results in the risk assessment and to contrast them with *in vivo* results. This is applicable even if not all inconsistencies can be resolved because of the constantly evolving nature of methods and our understanding of results. The new IATA methods could potentially facilitate the evaluation of the effect of complex mixtures and provide information on pathway-based changes that should offer a better understanding of biological plausibility for epidemiological studies.

Notwithstanding the limitations of traditional epidemiological studies, the inherent uncertainties in the existing testing paradigm point to a clear need to collect information in a disciplined and orderly way in order to follow up on adverse effects that cannot always be reliably predicted during pre-market toxicity tests. To this end, there are several large ongoing studies that may provide new insights into the effects of pesticides. This includes the U.S. Agricultural Health Study (AHS), which is a large long-term prospective study that has released a number of preliminary publications evaluating both cancer and non-cancer outcomes in licensed pesticide applicators.[48] Additionally, over the next several years the AHS will

48 U.S. Agricultural Health Study: http://aghealth.nci.nih.gov/

evaluate the impact of genetic and other biological risk factors on epidemiological outcomes. Although still in its early phases, the National Children's Study will examine the effects of environmental influences (including pesticide exposures) on the health and development of more than 100,000 children across the United States, following them from before birth until age 21 years.[49] In anticipation of the growing epidemiological evidence, it is important to establish well-defined procedures for incorporating and integrating epidemiological information into pesticide risk assessments and that appropriately qualified staff are recruited by regulatory agencies in order to capture various aspects of epidemiological studies. The U.S. *Federal Insecticide, Fungicide, and Rodenticide Act* (FIFRA) (U.S. Government, 1972b) recently provided specific guidance on incorporating epidemiology into pesticide risk assessment (US EPA, 2010b); these guidelines serve as a useful resource in developing a defined procedure or framework.

The Challenge of Post-Marketing Surveillance for Environmental Chemicals:

Laboratory toxicity testing is used because safety assessment in human populations is neither practical nor ethical. As such, and regardless of the technologies used, toxicity testing is essentially an exercise in predictive modelling. In the context of environmental chemicals, post-market surveillance represents a practical means of verifying whether these predictions are correct and may provide a safety net if they are not. Surveillance is defined as "the ongoing, systematic collection, analysis, and interpretation of health data essential to the planning, implementation, and evaluation of public health practice, closely integrated with the timely dissemination of these data to those responsible for prevention and control" (Langmuir, 1963). Post-market surveillance of populations exposed to new pesticides may provide a means of identifying unanticipated health effects at lower levels of exposure and in susceptible or high-risk groups. In addition, it may also facilitate a quantitative analysis of pre-marketing exposure predictions in different subpopulations and by different routes of exposure.

As mentioned above, there is currently no formal program of post-market surveillance for pesticides in Canada; therefore, the identification of unanticipated health effects has relied on passive mechanisms. The largest source of information on pesticide poisonings in Canada is from the Poison Control Centres, which deal almost exclusively with acute accidental poisonings. All provincial poison control agencies collect information on pesticide poisonings, and some jurisdictions, such as the province of Quebec (Gouvernement de Québec, 1998), require physicians and public health nurses to report instances of pesticide poisoning. In addition, pesticide registrants are required to report incidents to the PMRA, and several hundred of these are received each year (PMRA, 2008a). Although these passive

49 National Children's Study: http://www.nationalchildrensstudy.gov/Pages/default.aspx

systems provide a means to identify acute adverse health outcomes, they represent a very limited form of surveillance because they are only applicable in cases where pesticide exposure can be identified (either by the patient or health-care provider) as the cause. This approach is unable to identify changes in the incidence of chronic disease or provide data on the number of exposed individuals.

Post-market surveillance of new chemical pesticides is a critical prerequisite for detecting additional safety information that cannot realistically be collected during the pre-market approval process and for conditions that only manifest after chronic exposure (Schroeder, 1998). This is true for both pesticides and other categories of chemicals. Indeed, much can be learned about the utility and importance of post-market surveillance from its application in pharmaceutical medicine, where it represents a crucial component of the regulatory process (Box 2.7). Pharmaceutical drugs are evaluated, pre-market, through a series of preclinical and clinical trials, which culminate with Phase III trials that are conducted with large patient groups (the size of Phase III trials vary from several hundred to several thousand individuals) with the goal of definitively evaluating the safety and efficacy of a drug. Nonetheless, pre-market trials frequently lack the statistical power to accurately capture rare adverse events, particularly those that occur at a frequency of 1 in 10,000 or lower, or that disproportionately affect sensitive subpopulations (Anello & O'Neill, 1996; Brewer & Colditz, 1999). As a result, it is only after drugs with uncommon adverse effects are introduced into the market and administered to many thousands or hundreds of thousands of patients that a rare adverse event can be statistically verified.

Although there are similarities between pesticide- and drug-mediated adverse events, there are also important differences that impact the ease with which biomonitoring data can be collected and interpreted. Pharmaceutical drugs are administered in defined doses, at prescribed intervals — up to 50 milligrams per day for Rofecoxib (Ross *et al.*, 2009; Sommet *et al.*, 2008) — for specific periods of time, and typically under the supervision of a physician. These kinds of controlled conditions are rarely (if ever) applicable to environmental chemicals. Even in the case of agricultural workers, for whom pesticide exposures are intermittent and minimized by the use of safety equipment and clothing, exposures are not to a prescribed concentration on a daily basis. More typically, humans experience prolonged and intermittent exposures to low or trace amounts of mixtures through environmental contaminants, including exposure to pesticide residues from various sources (including food). This makes establishing a causal linkage between exposure and adverse health outcomes extremely challenging, and highlights the need for effective post-market surveillance for both new pesticides and new drugs (although the Panel acknowledges that the challenges in doing this are very different).

Box 2.7
CASE STUDY: Post-Market Surveillance

Post-market surveillance of chemical exposure is fraught with difficulties including insight into exposure, availability of clinical markers of effect, and the number of people exposed for statistical interpretation. Often measures of pesticide exposure are imprecise, clinical markers of effect are absent, and the number of people exposed are limited. Unlike pesticides, data are more robust for prescription medications such as Rofecoxib (Vioxx).

Rofecoxib was introduced onto the market in May 1999 and became widely prescribed for the acute and chronic treatment of pain, inflammation, and arthritis. Vioxx was withdrawn from the world market on 30 September 2004, after it had been shown to increase the risk for stroke and heart attacks (Juni *et al*., 2004). The FDA database used to assess the safety of Rofecoxib included only about 5,000 patients, and at that time there was no indication of a significant increased risk for heart attack or stroke (Kweder, 2004). The inability to predict such uncommon adverse outcomes in advance is due to the limited number of patients and limited duration of treatment during pre-market testing.

Retrospective analysis of placebo-controlled clinical trial data for Rofecoxib and of data for this drug in the French pharmacovigilance database demonstrated that drug administration was associated with an increased risk for a cardiovascular adverse event or death (Ross *et al*., 2009; Sommet *et al*., 2008). The drug was also associated with thrombotic adverse drug reactions.[50]

Such studies show the need for effective post-market surveillance, especially for low-intensity effects that occur as a result of chronic exposure.

The Challenge of Addressing Intraspecies Variability:

As concern for vulnerable groups within populations has grown, susceptibility has become an important element in the risk assessment and risk management process. Susceptibility is the extent to which the response to exposure is affected by factors such as genetics, age, pre-existing conditions, and behaviour.

50 The Panel recognizes that there were other factors that contributed to the adverse effects observed with Rofecoxib (e.g., misrepresentation of data); however, the statistical problems inherent in identifying low incidence effects as a result of chronic exposure make it a powerful example of the value of post-market surveillance.

The impact of inter-individual variability in both the manifestation of adverse drug reactions and in differences in the clinical response to drug treatment has been acknowledged for many years. This variability is attributed to a complex interplay between environmental and genetic factors (reviewed in Squassina *et al.*, 2010). By extension, it might be reasonable to assume that specific individuals within a population may be more susceptible to adverse outcomes as a result of exposure to environmental chemicals.

The genomes of individual humans are more than 99 per cent identical, but among the three billion DNA base pairs of the human genome there are over 10 million locations where sequences differ between individuals.[51] These differences in DNA sequence — collectively referred to as genetic variation — can lead to differences in individual responses to chemical exposure. In the context of pharmaceutical development and prescribing, this variation in chemical response forms the basis for pharmacogenetics and is the conceptual foundation for personalized medicine. In addition, epigenetic differences among individuals and populations, which may be inherited but also may vary with life stage and environmental exposures, have become increasingly appreciated as another cause of intraspecies variation. In the context of environmental toxicology, genetic and epigenetic variation might result in inter-individual variability in susceptibility to the adverse effects produced by chemical exposure.

Although the principles behind the adverse responses may be similar between pharmaceutical drugs and environmental chemicals, the challenge of establishing causation is quite different. Nevertheless, the data derived from pharmacogenetics studies hold tremendous promise for the evolution of quantitative biosurveillance studies based on a mechanistic understanding of disease etiology. The deployment of these studies could provide the scientific basis for establishing causative linkages between exposure and adverse health outcomes.

2.5.5 Inability to Effectively Evaluate Mechanisms of Toxicity

The existing approach to toxicity testing does not readily permit the identification and elucidation of mechanistic information. A mechanism of action specifically describes how a substance exerts its effect on the living system. The subsequent understanding is the basis for studies in mechanistic toxicology, in which the term "mechanism of action" denotes the sequence of events leading from the

51 Deoxyribonucleic acid (DNA) is composed of many nucleotides, each of which consists of a sugar phosphate backbone, and one of four bases (adenine, thymine, guanine, or cytosine). DNA encodes the heritable information for all living things.

absorption of an effective dose of a chemical to the production of a specific biological response in the target organ (as described in Box 2.4).[52]

Although a complete mechanistic understanding for every chemical and toxicity endpoint may not be realistic (or even necessary), understanding the MoA for an observed toxicological response can be invaluable in determining the relevance of an observed endpoint to humans. It could also provide a scientifically defensible means by which toxicity might be inferred based on structural similarities of related chemicals, which would be helpful in priority setting for data-poor chemicals.

The potential applications for this information are extensive, and a detailed discussion is beyond the scope of this report; however, in the long term, mechanistic information may help to address some of the issues discussed in this chapter as well as other emerging issues, for example, gender-specific and life-stage-specific responses as well as the impact of epigenetics.[53]

2.5.6 Consideration of Possible Endocrine Effects of Environmental Chemicals

Some chemicals, including certain pesticide active ingredients, have been shown to exhibit hormone-like activity and adversely affect endocrine homeostasis in animal experiments (H. R. Andersen *et al.*, 2002; Gray *et al.*, 2001; Grunfeld & Bonefeld-Jorgensen, 2004; Gutendorf & Westendorf, 2001; Hodges *et al.*, 2000; Kelce *et al.*, 1997; Kelce *et al.*, 1995; McLachlan *et al.*, 2006; Ramamoorthy, Wang, Chen, Norris *et al.*, 1997; Ramamoorthy, Wang, Chen, Safe *et al.*, 1997; Sharara *et al.*, 1998; Soto *et al.*, 1994; Wade *et al.*, 1997). Indeed, increased incidences of developmental abnormalities, combined with rising rates of several hormone-related diseases, have contributed to a growing concern that environmental contaminants may harm human health through the disruption of the endocrine system (reviewed in Damstra, 2003; Daston *et al.*, 2003; Foster *et al.*, 2004; McLachlan *et al.*, 2006; Phillips & Tanphaichitr, 2008). For example, reports of developmental abnormalities of the male reproductive tract (Chilvers, 1992; Chilvers *et al.*, 1984); rising rates of testicular cancer in young men (Skakkebaek *et al.*, 2001); reports in worldwide declining sperm counts (Carlsen *et al.*, 1992) (although controversial) and decreased semen quality (Almagor *et al.*, 2003;

52 The distinction between mode of action and mechanism of action is often confused in the toxicological literature. Mechanism of action describes a complete molecular understanding of the sequence of events from exposure to toxic outcome (Schlosser & Bogdanffy, 1999). Mode of action is a more general term; it refers to the type of response produced in the exposed subject or to the critical components of the mechanism that resulted in the observed biological response (Borgert *et al.*, 2004).

53 "Epigenetics" refers to heritable changes in phenotype through mechanisms other than changes in the DNA sequence.

Auger *et al.*, 1995; Bendvold, 1989; Feki *et al.*, 2009; Irvine *et al.*, 1996; Jorgensen *et al.*, 2002; Shine *et al.*, 2008; Younglai *et al.*, 1998);[54] and increased incidences of breast cancer in women (Bray *et al.*, 2004; Marshall, 2011; Parkin *et al.*, 2005; Smith *et al.*, 2009) add to concerns that human health may be adversely affected by exposure to environmental contaminants.

Furthermore, environmental contaminants may be involved in other hormonally dependent diseases — including hypothyroidism, diabetes, obesity, attention deficit and hyperkinetic disorder, endometriosis, and polycystic ovarian disease — raising concerns that these chemicals may play a role in mediating the documented changes in human health (Bourguignon & Parent, 2010; Foster *et al.*, 2004; Grun & Blumberg, 2009; Hotchkiss, *et al.*, 2008; McLachlan *et al.*, 2006).

The above examples indicate the potential existence of numerous pathways through which an environmental chemical in general (and pesticides in particular) may affect endocrine homeostasis in a way that might elicit profound effects on the development of the organism, its subsequent reproductive fitness, and general health in adulthood. While the field of endocrine toxicology continues to evolve, debate over the importance and relevance of some observations is unavoidable. For this reason, the screening and testing of pesticide active ingredients for potential endocrine-disrupting capacity has started to emerge within the pre-market testing requirements of some jurisdictions (discussed in more detail in Chapter 4).[55] Although it is unclear how the tests that are being developed as a result of these initiatives will be used in the Canadian regulatory process, it is worth noting that the PMRA does play a role in the development of the resultant guidelines via its work with the OECD. Despite these new initiatives, the breadth of physiological functions regulated by the endocrine system, together with unique characteristics of hormone signalling, further complicate the development of screening and testing strategies. Specifically, the endocrine system responds to changing environmental cues and regulates homeostasis by the action of hormone signals on growth, neurodevelopment, satiety, digestion and metabolism, lactation, stress (fight or flight response), and reproduction. Over the long term, as research in endocrine

54 Although it should also be noted that there are a number of studies that found no difference in semen quality (Andolz *et al.*, 1999; Larsen *et al.*, 1998; Paulsen *et al.*, 1996).

55 The United States Congress modified the *Food Quality Protection Act of 1996* and the *Safe Drinking Water Act* of 1974 to include the requirement for the testing of estrogenicity and other hormonal activity (United States Government, 1974, 1996a, 1996b). In response, the EPA developed a tiered screening and testing program for endocrine disruptors (specifically those affecting the estrogen, androgen, and thyroid hormone systems) (US EPA, 2009a). Similarly, the OECD developed the uterotrophic assay, the Hershberger assay, and the enhanced OECD test guideline 407 to the list of recommended tests (OECD, 2008b). In addition, the new Plant Protection Product legislation in the EU requires formal criteria for the identification of endocrine disruptors to be established by 2010 (European Union, 2009c).

toxicology continues to advance and novel endpoints and receptor-mediated signalling pathways are uncovered, the relevance of each response will need to be evaluated before it is considered for inclusion in future screening batteries.

2.5.7 The Value of Retrospective Analyses of Existing Testing Strategies

The current toxicity testing system to assess chemicals has been in place for several decades, and hundreds of chemicals have been evaluated under standard test guidelines. This information provides a rich database of existing toxicity information that can support the development of retrospective analyses. These analyses play an important role in supporting changes in data requirements, modifications to study designs, and improvements to prediction models.

A number of different retrospective analyses on various animal toxicity studies have recently been published. These analyses address issues that include the duration of toxicity studies that are appropriate for chronic risk assessment (Dellarco, Rowland *et al.*, 2010); the need for an additional test species for evaluating prenatal toxicity (Janer *et al.*, 2008); the additional information gained from the mouse cancer bioassay (Billington *et al.*, 2010); or the contribution of the F2 generation in rat reproductive studies (Janer, Hakkert, Piersma *et al.*, 2007; Janer, Hakkert, Slob *et al.*, 2007; Piersma *et al.*, 2011). Despite differences in interpretation — which may be explained by the differences in data sources, evaluation criteria, or chemicals included — the results of these studies serve to highlight the need for regular retrospective analyses of existing testing strategies in order to evaluate their suitability and ensure that lessons learned are reflected in future iterations. They also illustrate the importance of comprehensive data repositories for the storage, and retrieval, of legacy data.

Furthermore, the Agricultural Chemical Safety Assessment (ACSA) Technical Committee of the International Institute for Life Sciences (ILSI) Health and Environmental Sciences Institute (HESI) was established in 2000. Its role was to design an updated testing scheme for crop protection chemicals that incorporated the current understanding of toxicity and exposure science (Carmichael *et al.*, 2006). The committee published its findings in a series of papers that concluded that a tiered approach would be appropriate for the testing of agricultural chemicals including pesticide active ingredients (Barton *et al.*, 2006; Carmichael *et al.*, 2006; Cooper *et al.*, 2006; Doe *et al.*, 2006). The technical committee described how a tiered approach might evolve by gathering data on prognostic molecular markers, improved dose metrics, characterization of toxicity pathways, metabonomics, and system biological approaches (Doe *et al.*, 2006). They highlighted how computational toxicology approaches may be used to assist in the

design of studies by predicting potential adverse effects; these could then help to focus *in vivo* testing on relevant endpoints within the ACSA tiered approach. Doe *et al*. (2006) further noted that companies already use various screening assays before embarking on an expensive development program for a new chemical in order to increase confidence in a successful product. The ACSA tiered strategy might therefore represent a pragmatic starting point to bridge the transition from a one-size-fits-all *in vivo* battery approach to evaluations based on the mechanistic understanding of chemistry and biology.

2.5.8 Summary of the Current Regulatory System

The current testing requirements for pesticide active ingredients prescribe an extensive battery of tests designed to generate data on potential adverse effects for a wide range of endpoints, in different species, for different exposure durations, and over critical life stages and processes. Data from these tests are used to identify potential adverse effects and develop dose-response relationships that are integrated with modelled (or measured) estimates of exposure to serve as the basis for risk assessment for various pesticide-use scenarios.

Over the last several decades, the testing of pesticide active ingredients (particularly agricultural chemicals) has been extensive, has served the needs of risk managers, and has contributed significantly to our understanding of the toxicology of these products. Nonetheless, the testing scheme is expensive and time-consuming and cannot adequately evaluate the large numbers of chemicals with little or no available data. Furthermore, the relevance of the data from high-dose animal studies to human health outcomes is not well characterized and provides no insight into the mechanistic basis underlying the observed adverse outcomes. Even when the current testing approach is successful at defining the hazard, the information is usually of a specific nature, which may not be useful for extrapolation to other species, other life stages, or susceptible populations.

Today there are several new directives and initiatives for toxicity testing that reflect an increased demand for more toxicity information for those chemicals that are currently data-poor.[56] Continued reliance on an extensive battery of standard *in vivo* toxicology tests that use many animals, are costly to conduct, and are not necessarily good predictors of human toxicity will not be practical to address the large number of data-poor chemicals (including pesticide formulants and industrial chemicals) that need to be evaluated.

56 For example, REACH in the European Union (EU), the U.S. endocrine disruptor screening program, and the Canadian Chemicals Management Plan.

The issues inherent in the current approach are therefore two-fold. There is a need to address the lack of toxicity data for the vast majority of industrial chemicals (including pesticide formulants), coupled with the recognition that regulatory decisions should be made on the basis of the best available science.[57] As a result, there is a need for new approaches that are more predictive, more reliable, faster, less expensive, and provide mechanism-based information about the underlying toxicity of a chemical in order to inform human health risk assessment.

2.6 ADDRESSING THE LIMITATIONS: INTEGRATED APPROACHES TO TESTING AND ASSESSMENT

Over 30 years of experience bolster our level of comfort with the uncertainties — unknown or not yet identified or characterized — of the current *in vivo* approach to toxicity testing. Any alternative tests will have uncertainties; it will be important to characterize the nature of these uncertainties and objectively determine whether they are more or less acceptable than those of the tests they are designed to replace.

The Panel anticipates that uncertainties will remain a part of toxicity testing for the foreseeable future, regardless of the testing strategies used. The challenge will be to identify these uncertainties and develop science-based strategies to address them. This will necessitate adopting a long-term focus with short-term objectives. Advances in science should be used as the basis to improve toxicity testing in an iterative fashion, using biological understanding as the driver of change.

The science of toxicology has evolved rapidly since the current battery of animal studies were first put in place, and entirely new technologies are being developed that may be capable of replacing some of the older methods. There have been efforts to re-evaluate the current *in vivo* animal testing paradigm and design approaches that provide better and more efficient safety evaluations. These evaluations would be more responsive to the needs of both risk assessment and risk management (for example, see Carmichael *et al.*, 2006; NRC, 2006b; NRC, 2007).

Building on advances in information sciences, biology (molecular, cellular, and systems), and reliable high-throughput screening assays pioneered in the drug discovery field, toxicology is poised to transform itself into a science able to efficiently determine the biological pathways by which chemicals are capable of exerting adverse health effects. This will help evaluate more substances and

57 Note that it might not be possible to achieve both objectives with a single strategy. An approach that makes it possible to test large numbers of chemicals may not provide a more accurate method for data-rich chemicals.

provide a better understanding of the intrinsic toxicological properties of different chemicals. Besides application to individual chemicals, these new approaches will also enable new methods for assessing the effects of combinations of chemicals, and new ways of characterizing exposures. This transformation was promoted by the National Research Council in its 2007 report on *Toxicity Testing in the 21st Century: A Vision and a Strategy* (NRC, 2007).

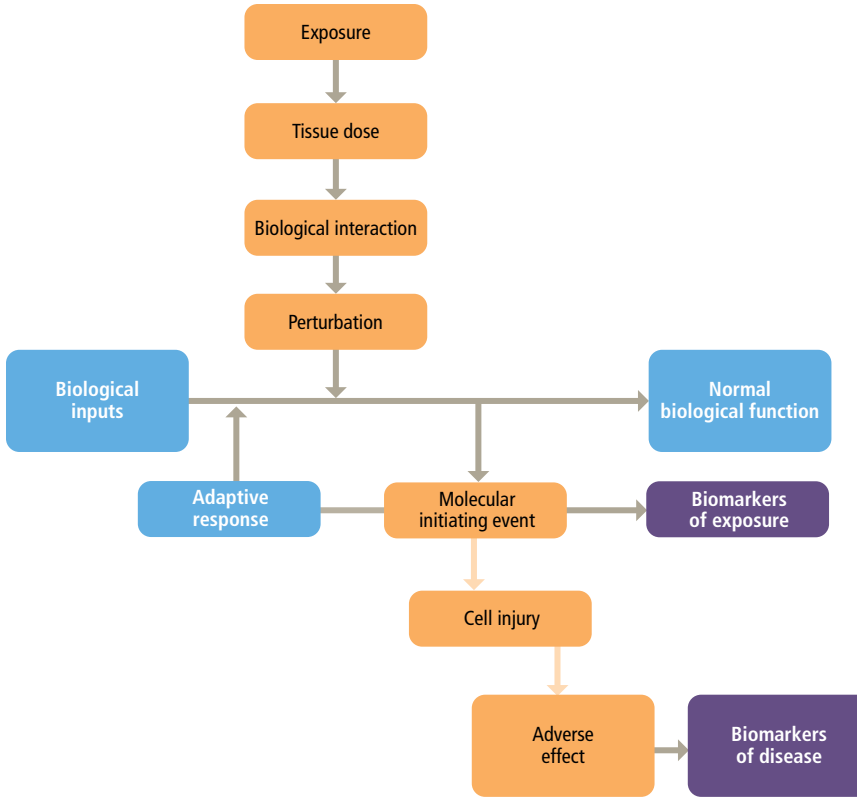
The 2007 NRC report describes a future in which all toxicity testing for regulatory risk assessment is conducted *in vitro*, using assays that have been designed to test the potential of a chemical (or mixture of chemicals) to cause biologically significant perturbations of key "toxicity pathways" (Figure 2.4) (NRC, 2007).[58]

The development of assays based on the perturbation of key toxicity pathways presupposes a comprehensive understanding of human physiology. Such an understanding would permit extrapolation of cellular responses to the organismal level. This systems-level understanding of human biology does not yet exist; however, considerable advances are being made in the emerging field of systems biology (Figure 2.5). If the vision espoused by the NRC report is that of understanding the perturbations of individual pathways that contribute to an adverse health outcome, systems biology is the vehicle that will permit extrapolating from cellular perturbation to adverse health outcome.

It is important to note that the vision for toxicity testing espoused by the 2007 NRC report will take many years to come to fruition. Considerable work needs to be done to address the scientific limitations of an *in vitro-in silico* approach, and fundamental, philosophical changes in the risk assessment paradigm may also be necessary for the data generated by these tests to be useful in a regulatory context. In the meantime, the Panel believes that there are many areas of toxicity testing that can be improved by integrating alternative approaches (including *in vivo*, *in vitro*, and *in silico* assays) into the current regulatory paradigm. It is on these advances that the Panel has elected to focus its attention.

The following chapters serve to outline an integrated approach to toxicity testing that could help bridge the transition from a rigid and mainly *in vivo* system to one that can embrace and adapt to new scientific advances. This approach would permit regulators to move away from a checklist style of approach and towards a knowledge-based assessment that could integrate all existing knowledge of a

58 The committee responsible for the 2007 NRC report, *Toxicity Testing in the 21st Century: A Vision and a Strategy*, defined "toxicity pathway" as a cellular response pathway that, when sufficiently perturbed, would be expected to result in adverse health effects (NRC, 2007).



(Adapted from Andersen, 2005 and reproduced with permission from Elsevier)*

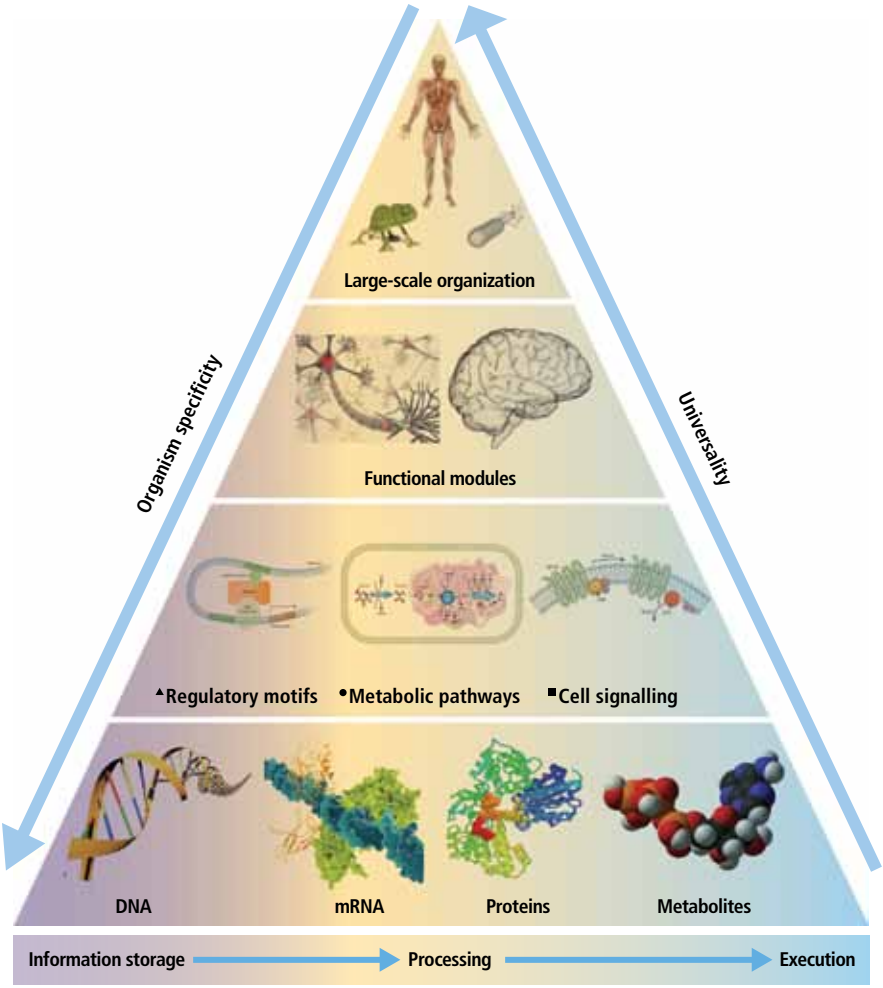
Figure 2.4

Exposure to chemicals can lead to perturbations of cellular pathways

When the extent of these perturbations exceeds the capacity of the adaptive responses to restore physiological homeostasis, adverse outcomes (may) arise.

*Reproduced from: *Trends in Biotechnology*, 23/3, Melvin E. Andersen, James E. Dennison, Russell S. Thomas, Rory B. Conolly, New directions in incidence-dose modeling, 122-127, 2005, with permission from Elsevier.

chemical into the risk assessment. This approach would also permit the integration of post-market epidemiological data into a pre-market screen of structurally related chemicals, providing regulators with an opportunity to consider population surveillance data in a way that has not been possible before.



(Adapted and reproduced with permission from AAAS)*

Figure 2.5

Systems biology integrates data across all levels of biological organization

Systems biology is the iterative and integrated study of biological systems across all levels of biological complexity, from molecular to organismal. It seeks to contextualize individual components in relation to their role as part of the entire organism. Arguably, systems biology is not a new concept, but recent advances in molecular biology and computer science have provided the technological capacity necessary to make the development of a systems-level understanding conceivable.

*From Oltvai, Z. N., & Barabasi, A. L. (2002). Systems biology. Life's complexity pyramid. *Science, 298*(5594), 763-764. Reproduced with permission from AAAS.

3

Tools and Data Sources Associated with Integrated Testing Strategies

- An Introduction to Integrated Approaches to Testing and Assessment
- The State of the Science of Alternative Testing Approaches
- The State of the Science of Alternative Testing Tools and Data Sources
- Scientific Challenges and Research Opportunities
- Transitioning to the Future

3 Tools and Data Sources Associated with Integrated Testing Strategies

What is the State of the Science of the Tools and Data Sources Associated with Integrated Testing Strategies?

LIST OF KEY TERMS*
Adaptive Response:
Changes that occur (typically in response to exposure) that permit a return to the normal (homeostatic) state without any irreversible disruptions to the overall system.
Adverse Outcome Pathway (AOP):
The sequence of events from chemical structure through the molecular initiating event to the *in vivo* outcome of interest.
Adverse Response:
Changes that occur that result in impairment of functional capacity, often due to an insult that exceeds the capacity of the adaptive response to permit a return to the homeostatic state. Outcomes might include changes in morphology, development, lifespan, or growth of the organism. Although harder to define at the molecular level, potentially adverse responses might include alterations in gene expression, protein synthesis, or cell cycle regulation.
Applicability Domain:
The physicochemical, structural, or biological space and information that was used to develop a (Q)SAR model, and for which that model gives predictions with a given level of reliability (Netzeva *et al*., 2005).
Assay (Bioassay):
A form of scientific experiment. The experimental process for determining the effects of a test substance on a biological system.
Bioavailability:
The extent to which a substance is absorbed into the systemic circulation of an organism. Bioavailability differs depending on route of exposure (e.g., intravenous administration is assumed to result in complete bioavailability). Bioavailability declines when exposure is mediated via other routes (e.g., oral, topical, etc.).
Biochemical Pathway:
A series of reactions, typically enzyme-catalyzed, that are associated with a specific physiological event in a living organism.
Cell Line:
Cells of a single type (human, animal, or plant) that have been adapted to grow continuously in the laboratory and are used in research.
Dose-Response Relationship:
The relationship between the amount of a substance to which an organism is exposed (i.e., the dose) and the magnitude of the observed adverse response.

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* Key terms as used by the Panel throughout this report. Additional terms are listed in the Technical Glossary in Appendix A.

LIST OF KEY TERMS *(continued)*
Ecotoxicology:
The study of the toxicology applied to all living organisms, including the effects on ecosystems, communities, and populations.
Enantiomers:
A pair of stereoisomers that are non-superimposable mirror images of each other.
Genotoxicity:
The degree to which an agent causes damage to genetic material.
High-Throughput Screening (HTS):
An approach that uses automated tools to facilitate the rapid execution of hundreds of thousands of assays per day in order to identify chemicals of concern for subsequent testing.
High-to-low-dose Extrapolation Modelling:
The process of predicting low exposure risk to humans and animals based on high-exposure, high-risk data obtained from laboratory animals.
Hypothesis-driven:
Approaches to science may be generalized as either descriptive or hypothesis-driven. Hypothesis-driven approaches are those that start by defining the key components that characterize the endpoint(s) of interest. In the context of toxicity testing, a hypothesis-driven approach begins by examining the chemical of interest in order to identify structural characteristics that confer toxicological potential. Subsequent steps narrow the focus to specific toxicity endpoints based on a mechanistic understanding of the interactions between the chemical and biological system.
Informatics:
An interdisciplinary field that studies the analysis, collection, classification, digitization, dissemination, manipulation, storage, and retrieval of data. Sub-disciplines are concerned with data from biological (bioinformatics) and chemical (chemoinformatics) sources.
Integrated Approaches to Testing and Assessment (IATA):
A tiered approach to data-gathering, testing, and assessment that integrates different types of data (including physicochemical and other chemical properties as well as *in vitro* and *in vivo* toxicity data). When combined with estimates of exposure in an appropriate manner, the IATA provides predictions of risk. In an IATA, unsuitable substances are screened out early in the process. This reduces the number of substances that are subjected to the complete suite of regulatory tests. Plausible and testable hypotheses are formulated based on existing information and/or information derived from lower tier testing and only targeted testing is performed in the higher tiers. Failure to satisfy the toxicity requirements at a lower tier typically precludes further testing at a higher tier.
Interactome:
All of the interactions between the biological constituents of a system.

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LIST OF KEY TERMS *(continued)*
Macromolecule:
A large and complex molecule. In biochemistry, these include nucleic acids (RNA and DNA), proteins, carbohydrates, and lipids as well as non-polymeric substances of large molecular mass.
Mode of Action (MoA):
The sequence of key cellular and biochemical events (measurable parameters), starting with the interaction of an agent with the target cell, through functional and anatomical changes, resulting in cancer or other adverse health effects. Mode of action differs from mechanism of action in that the latter implies a more detailed understanding of causality leading to an adverse outcome.[59]
Molarity:
The concentration of a substance in solution, expressed as the number of moles of solute per litre of solution.
Omics:
A term used to encompass fields of biological study that end in –omics. These include genomics, proteomics, metabolomics, transcriptomics, and toxicogenomics. In molecular biology, the suffix –ome is typically used to describe fields that consider constituent components collectively as part of a larger system. For example, the application of genomics technologies (including HTS assays) to the field of toxicology is termed toxicogenomics.
Perturbation:
A change in the biological system in response to exposure to a given substance.
Pharmacokinetics:
The study of the process by which a substance is absorbed, distributed, metabolized, and excreted by a biological system. Pharmacokinetics can be used to establish quantitative relationships between dose, concentration, and time.
Physicochemical Properties:
The physical and chemical characteristics of a substance.
Predictivity:
The prognostic power of a test as defined by its relevance and reliability to predict an outcome in humans.
(Quantitative) Structure-Activity Relationship ((Q)SAR):
A mathematical relationship that (quantitatively) links chemical structure and physicochemical properties to a well-defined process, such as biological activity or reactivity.

continued on next page

59 There are numerous definitions of Mode of Action and Mechanism of Action. The definition that the Panel is using may be found in Seed *et al.* (2005).

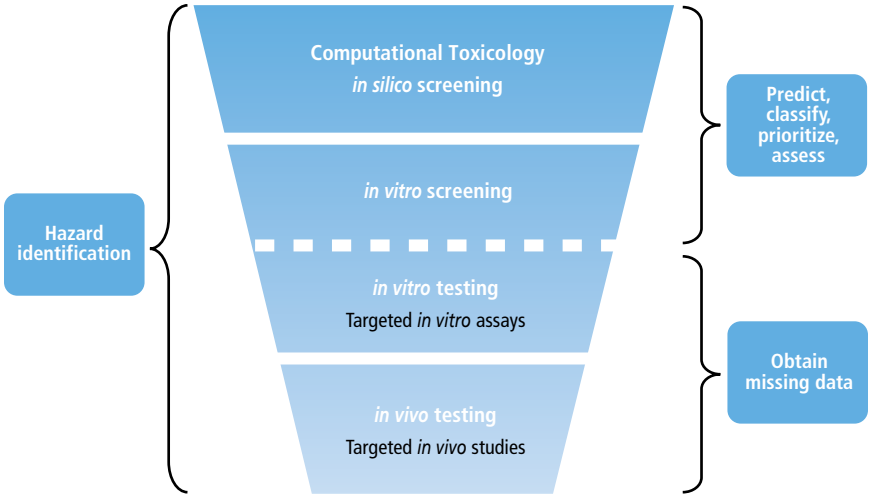
LIST OF KEY TERMS *(continued)*
Toxicity Pathway:
A cellular response pathway that, when sufficiently perturbed, would be expected to result in adverse health effects (NRC, 2007).
Toxicity Test:
An experimental approach designed to generate specific toxicity data on a chemical in order to characterize its intrinsic toxicological properties.

3.1 AN INTRODUCTION TO INTEGRATED APPROACHES TO TESTING AND ASSESSMENT

Integrated Approaches to Testing and Assessment (IATA) represent a hypothesis-driven strategy that incorporates new scientific advancements into the existing toxicity testing system in a transparent and scientifically credible manner. Using a tiered approach, IATA seeks to harness all the available data on a given chemical before the initiation of a toxicity testing battery in order to focus subsequent testing on the relevant endpoints of concern (Figure 3.1). As such, IATA focuses on finite testing resources to chemicals and endpoints of highest concern to human and environmental health. In some cases, this means identifying the toxicity endpoints of concern and targeting *in vivo* tests to these particular endpoints. In other cases, there may be adequate information available from higher tiers, mitigating the needs for any *in vivo* testing (Combes *et al.*, 2003). However, it should be noted that, although similarities exist between IATA and Integrated Testing Strategies (ITS), they represent distinct approaches (Box 3.1).

Box 3.1
A Brief Aside on IATA Versus ITS

Both Integrated Approaches to Testing and Assessment (IATA) and Integrated Testing Strategies (ITS) are pragmatic approaches to bridge the transition from the current one-size-fits-all *in vivo* testing to assessments that are based on a mechanistic understanding of both chemistry and biology. Nonetheless, IATA and ITS are fundamentally different approaches. IATA adopts a tiered approach in which failure to satisfy the structural or toxicity requirements at a lower tier would typically rule out further testing at a higher tier, whereas ITS is a data-gathering and testing scheme (Jaworska & Hoffmann, 2010).



(Adapted and reproduced with permission from Taylor & Francis Group and Dellarco, Henry *et al.*, 2010)*

Figure 3.1

The IATA approach

The purpose of an IATA approach is to focus testing on the endpoints of concern. This is done by harnessing existing knowledge to focus research on answering those questions that to date could not be addressed.

*Meeting the Common Needs of a More Effective and Efficient Testing and Assessment Paradigm for Chemical Risk Management, Vicki Dellarco, Tala Henry, Phil Sayre *et al.*, *Journal of Toxicology and Environmental Health, Part B: Critical Reviews*, 2010, Taylor & Francis, reproduced with permission of the publisher (Taylor & Francis Group, http://www.informaworld.com).

IATA relies on various tools and techniques in order to target testing to the chemicals and endpoints of highest concern. This requires a broad set of existing data (both toxicological and physicochemical) and a scientifically robust and systematic means of evaluating it in order to accomplish the following objectives:

- To adopt a hierarchical, intelligent, risk-based approach rather than reliance on a hazard-based checklist of tests;
- To facilitate a proper consideration of exposure as a key component in risk assessment;
- To integrate state-of-the art approaches in a transparent and scientifically defensible manner;
- To use the best available data from different sources;
- To reduce uncertainty by gaining knowledge of biological interactions;

- To permit the faster evaluation of a greater number of chemicals across a broader range of potential endpoints; and
- To use a tiered approach to help categorize and prioritize higher risk chemicals.

The strength of IATA lies in the breadth of information that is used to understand the toxicological profile of a chemical. This understanding is ultimately used to inform a regulatory decision. While the specific components of an IATA approach may differ depending on the nature of the chemical in question, the regulatory domain of interest, the available existing data, and the proposed usage of the final product, there are some common components inherent to any IATA-driven approach (Table 3.1 and Figure 3.1).

Table 3.1

Essential components of an IATA approach designed to inform subsequent risk assessment

Purpose	Types of Information	Tools and Data
To predict (human) exposure	• Assessment of physiochemical properties • Predicted exposure • Biodegradability	• All existing physicochemical data • Proposed uses and user information • Existing exposure information • Chemical categorization • Post-market surveillance and human exposure studies for relevant agents
To classify the chemical and prioritize for subsequent testing	• Predicted toxicity • Estimated exposure levels • Predicted metabolic breakdown products	• All available hazard data • SAR and (Q)SAR • Computational predictions of toxicity • Read-across • All existing toxicity information • High-throughput screening • Expert judgment • PBPK models
To assess additional data needs	• Identified data gaps • Identification of toxicity endpoints needed for risk decision	
To obtain missing data	• Toxicity data on specific endpoints of concern	• Targeted *in vitro*, *in silico*, and *in vivo* tests

(Adapted from Combes, *et al.*, 2003)

As shown in Figure 3.1, an IATA strategy is hierarchical. Early screening facilitates the rapid evaluation of a large number of substances in order to identify chemicals of concern. Subsequent steps identify data gaps for further (targeted) testing. The initial screens rely primarily on *in silico* modelling using existing data (where available) and data generated through the application of *in vitro* high-throughput

screening (HTS) assays. Targeted testing uses both *in vitro* and *in vivo* approaches to provide the necessary toxicity data in order to permit hazard identification and inform a subsequent risk assessment. The integration of these approaches makes IATA a powerful and potentially transformational paradigm for regulatory toxicity testing.

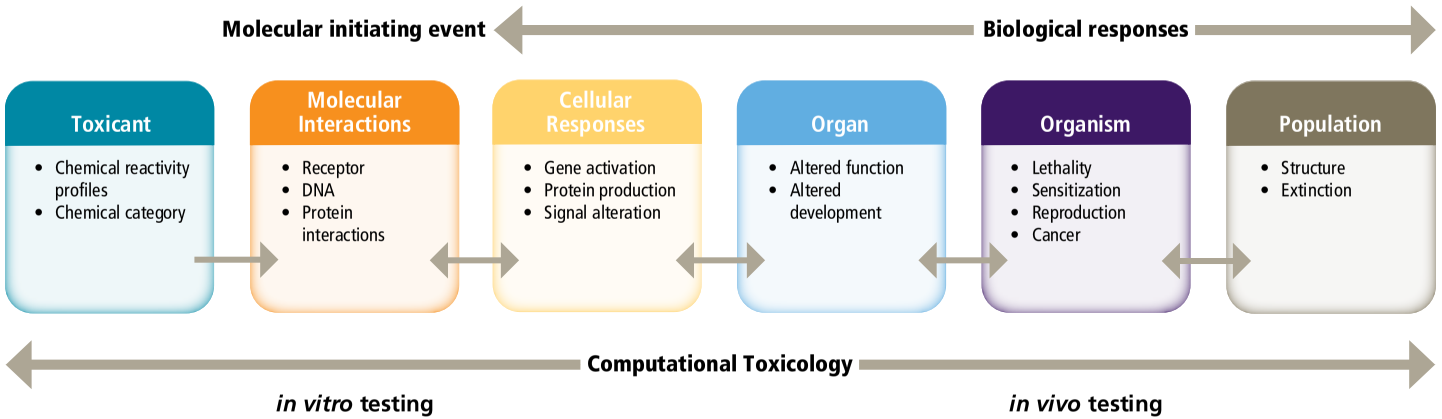
This chapter will describe the state of the science that ultimately informs the development of new toxicity testing approaches. It will also examine current state-of-the-art approaches that leverage existing data to screen and prioritize chemicals. It will conclude with a summary of the scientific challenges and research opportunities relevant to a transition towards a more integrated approach to testing and assessment.

3.2 THE STATE OF THE SCIENCE OF ALTERNATIVE TESTING APPROACHES

A toxicological endpoint describes the result of an interaction between the physicochemical properties of a chemical agent and the biology of the target organism (US EPA, 2005). It is an empirical observation of *what* happened. In contrast, an approach based on a mode of action (MoA) or adverse outcome pathway (AOP) describes the physiological basis for the toxicological effect. This approach refers to the type of response, including the key steps elicited in the target organism needed to cause the biological response (Borgert *et al.*, 2004). As such, the MoA and AOP describe *how* it happened.[60]

A multitude of disciplines will contribute to elucidating MoAs and AOPs. At the heart of this highly complex and interdisciplinary research landscape are the fields of systems biology and computational toxicology. Although a complete mechanistic understanding of the biological responses underlying most toxicity outcomes is arguably many years in the future, work is underway to develop this kind of biological systems-level understanding. The Panel believes that an IATA approach could be used to integrate incremental advances and augment the regulatory process.

60 The mechanism of action of a chemical describes the complete molecular sequence of events from exposure to the manifestation of the toxicological outcome. In contrast, a MoA describes key events in the pathway but does not necessitate a complete elucidation of the molecular sequence.



(Adapted from Ankley *et al.*, 2010 & US EPA, 2010c and reproduced with permission from John Wiley and Sons)

Figure 3.2

An adverse outcome pathway associates exposure to a toxicant with outcomes at the population level through ascending levels of biological complexity

3.2.1 The Adverse Outcome Pathway (AOP)

An AOP may be defined as the sequence of events from chemical structure through the molecular initiating event to the *in vivo* outcome of interest (Figure 3.2). Each AOP represents a set of responses that characterize the cascade of biological effects caused by a particular molecular initiating event (Schultz, 2010).[61] While AOPs often start out being depicted as linear processes, the amount of detail and linear character of the pathway can vary significantly, especially for chronic health endpoints. Nonetheless, although a number of biochemical steps are required for a toxic response to be realized, the molecular initiating event is a prerequisite for all subsequent steps (Enoch & Cronin, 2010).

Although developed for use in ecotoxicology (Box 3.2), AOPs depict the relationship between a molecular initiating event and an adverse outcome at the individual or population level. As such, they are directly applicable to human health endpoints (Ankley *et al*., 2010; Bradbury *et al*., 2004). AOPs seek to group chemicals based on both up-stream chemical and down-stream biological processes. As a result, they delineate the documented, plausible, and testable processes by which a chemical-induced molecular perturbation may elicit an effect (or effects) at the subcellular, cellular, tissue, organ, whole-animal, and population levels.[62]

An important aspect of quantifying an AOP is the threshold and scale of the linkage between key events in the pathway. For example, the threshold of estrogen receptor (ER) binding required to elicit vitellogenin synthesis in the liver, and the blood concentration of vitellogenin indicative of estrogen exposure sufficient to cause gonadal conversion (Box 3.3).[63] Confidence in an AOP is increased by a more comprehensive understanding of the nature of the interaction between the chemical and the biological system, coupled with a mechanistic understanding of the biological response.

61 Earlier descriptions of an AOP referred to it as a "toxicity pathway." The term adverse outcome pathway was adopted following the release of the 2007 NRC report *Toxicity Testing in the 21st Century: A Vision and a Strategy* to mitigate the potential for confusion. An AOP differs from the NRC's "toxicity pathway" concept (NRC, 2007), which is primarily cell-based (although it uses knowledge that comes from tissue-level studies). The AOP more explicitly includes the progression of events from the molecular to the population level.

62 AOPs are based on chemical interactions at the molecular level. Because adverse effects observed *in vivo* are a function of both the chemical structure of the toxicant and the result of many biological responses, AOPs are designed to avoid mixing data from different molecular initiating events that can cause the same *in vivo* outcome.

63 Although vitellogenin is a female-specific protein, exposure to exogenous estrogens can induce its synthesis in males; therefore, the existence of vitellogenin in blood can be used as a biomarker for environmental estrogens.

Box 3.2
Adverse Outcome Pathways in Ecotoxicology and Environmental Risk Assessment

Besides studying changes at the subcellular-, cellular-, tissue-, organ- and organism-levels of biological organization, ecotoxicology must also study the effects at the population level resulting from exposure to a toxicant.

The number and variety of possible interactions increases significantly as the levels of biological complexity increase. Using the traditional approach to toxicity testing to test all chemicals for their potential adverse effects on ecosystems is neither practical nor feasible. Although in theory environmental risk assessment deals with millions of species, in practice a limited number of assays are used to study effects in a representative number of taxonomic groups. This reality has necessitated the development of scientifically robust tools and models that can be used to predict adverse effects of chemicals using limited primary data.

The AOP approach evolved from previous conceptual frameworks (Ankley *et al*., 2010; Schultz *et al*., 2006). The biological foundation can be traced to the mode of action studies of McKim *et al*. (1987). Their fish acute toxicity syndromes are represented by selected biochemical or physiological effects of exposure. These syndromes, selected as key responses, were measured *in vivo* from exposure to model chemicals (Bradbury *et al*., 1990).

The AOP approach has been described as an organizing framework to help facilitate environmental risk assessments for toxic chemicals (Ankley *et al*., 2010). By considering the entire process from exposure through to the effect at the population level, AOPs provide a pragmatic means of applying diverse ecotoxicological information from different levels of biological complexity to a risk assessment.

Box 3.3
CASE STUDY: AOPs and Phenolic Estrogen Mimics in Fish

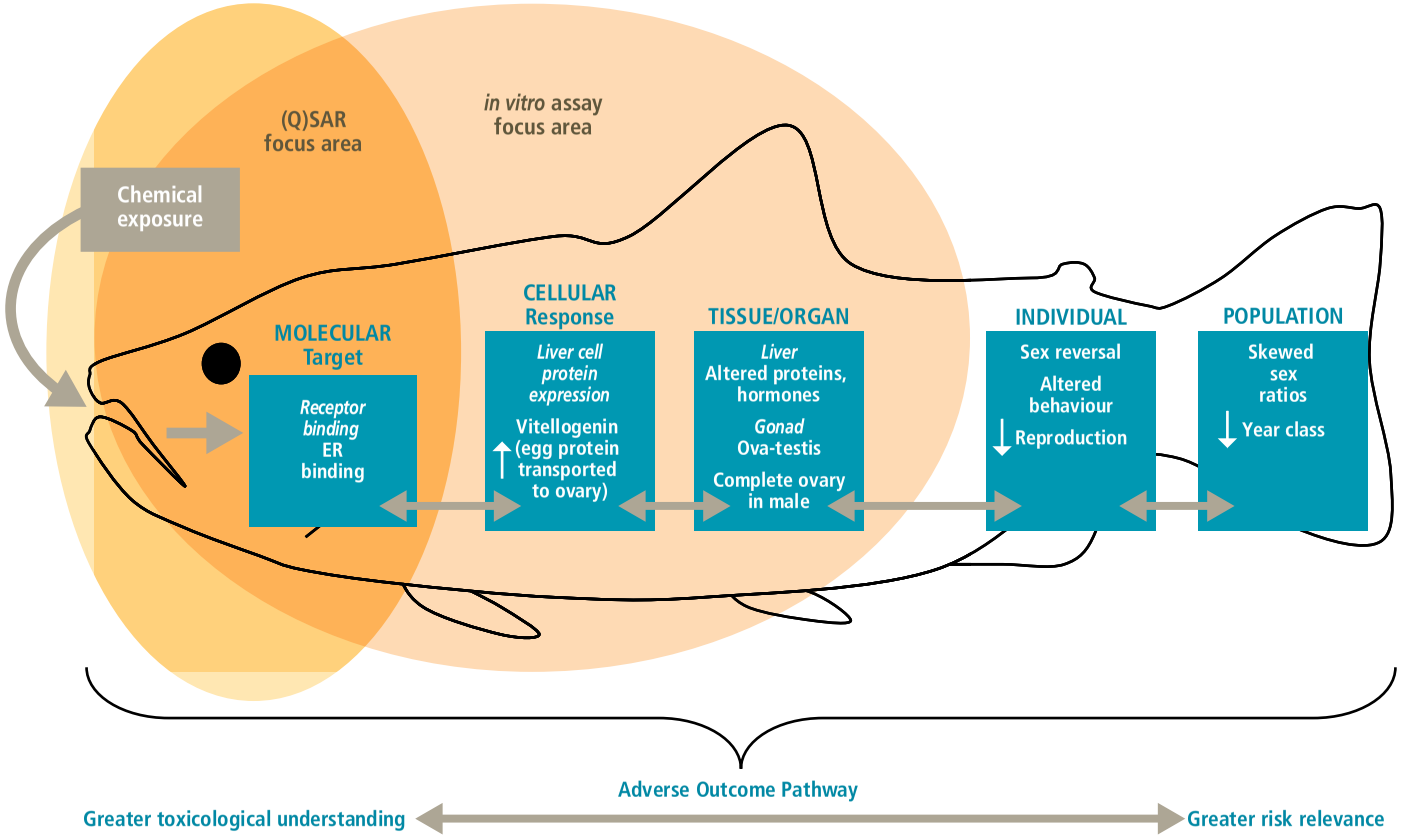
Endocrine disruption has been linked to ER binding by environmental chemicals that include industrial chemicals, phytoestrogens, and steroid hormones (OECD, 2009j). The reproductive impairment of fish after exposure to phenolic compounds represents a particularly well-documented example that has led to the establishment of an AOP (OECD, 2009j; Schmieder *et al*., 2004). This AOP is illustrated in Figure 3.3 and can be summarized as follows:

- Binding of the chemical to the A-site of the ER is the molecular initiating event.
- The affected biochemical pathways are hormonally linked; the resulting perturbations are reversible.
- The cellular-level consequence is an up-regulation of estrogen-responsive gene transcription.
- There are multiple target organs, including the liver and gonads.
- The biological response to the cellular effects is induction of vitellogenin synthesis in the liver and conversion of testicular tissue to ova tissue.
- The organismal response to the biochemical, cellular, and biological effects is the feminization of male fish.
- The overall effect on the fish population is reproductive impairment.

ER binding-induced reproductive impairment in fish results in several measurable events, some of which are represented in databases of sufficient size and diversity to demonstrate their importance in the AOP. Measurable events along the pathway include:

- Estrogen binding, measured quantitatively in competitive binding assays using radiolabelled [^{3}H]-17-β-estradiol (Schmieder *et al*., 2004).
- Vitellogenin induction, measured using real-time RT-PCR for vitellogenin mRNA using the fish liver tissue slice assay (Schmieder *et al*., 2004).[64]
- Conversion of testicular tissue to ova tissue, measured morphometrically (from histological section) using the Medaka assay (Ankley & Johnson, 2004; Miller *et al*., 2007).

64 Real-time, reverse-transcription PCR is often denoted as qRT-PCR or RRT-PCR or RT-rt PCR. It describes an approach that combines real-time PCR with reverse transcription in order to quantitate mRNA in cells or tissues.



(Adapted and reproduced with permission from the American Chemical Society, 2004)

Figure 3.3

An adverse outcome pathway linking exposure to phenolic estrogen mimics to population-level outcomes in fish

3.2.2 The Mode of Action (MoA) Approach

Although a complete understanding of the molecular mechanisms by which a chemical elicits its observed effect may be rare, MoA data does exist for many chemicals (US EPA, 2005); this is especially true for data-rich compounds such as pesticides.[65] In these instances, regulatory initiatives have allowed scientifically valid MoA data to be used to inform a risk assessment (see Dellarco & Baetcke, 2005 for a review of some of these initiatives).

An MoA analysis determines how relevant animal-derived data is to humans. It also considers the influence of the aggregated information on dose-response extrapolation methods and default uncertainty factors. For any given chemical, there might be multiple MoAs that impact different toxicity endpoints; for any given toxicity endpoint, there might be alternative and relevant MoAs. The MoA approach is based on understanding the key events along a causal pathway that leads to a toxicological outcome. These key events must be supported by robust experimental and mechanistic data. There must also be a clear description of the confidence in the evaluation, identification of any data gaps, and a discussion of the implications for a subsequent risk assessment (Boobis *et al.*, 2008).

The International Programme on Chemical Safety (IPCS) recently published Human Relevance Frameworks that are intended to permit the systematic evaluation of toxicity data for cancer and non-cancer endpoints in a transparent and scientifically credible fashion (Box 3.4) (Boobis *et al.*, 2006; Boobis *et al.*, 2008). These weight-of-evidence (WoE) frameworks can apply to all data situations and are a valuable approach for identifying and providing clarity around critical data gaps.

The IPCS framework provides structure, scientific rigour, and transparency to the evaluation of MoA data. Both the IPCS MoA/human relevance framework and the OECD principles of (Q)SAR validation (OECD, 2007b) provide examples of transparent processes that evaluate, through the use of scientific criteria, the predicted results of an approach. The MoA framework also has significant value for the evaluation of data-poor chemicals, especially if an understanding of the MoA can be inferred from existing toxicity data on chemicals with shared properties.

65 Note that the pesticidal MoA may or may not be the same as the toxic MoA.

Box 3.4
CASE STUDY: The IPCS Human Relevance Frameworks

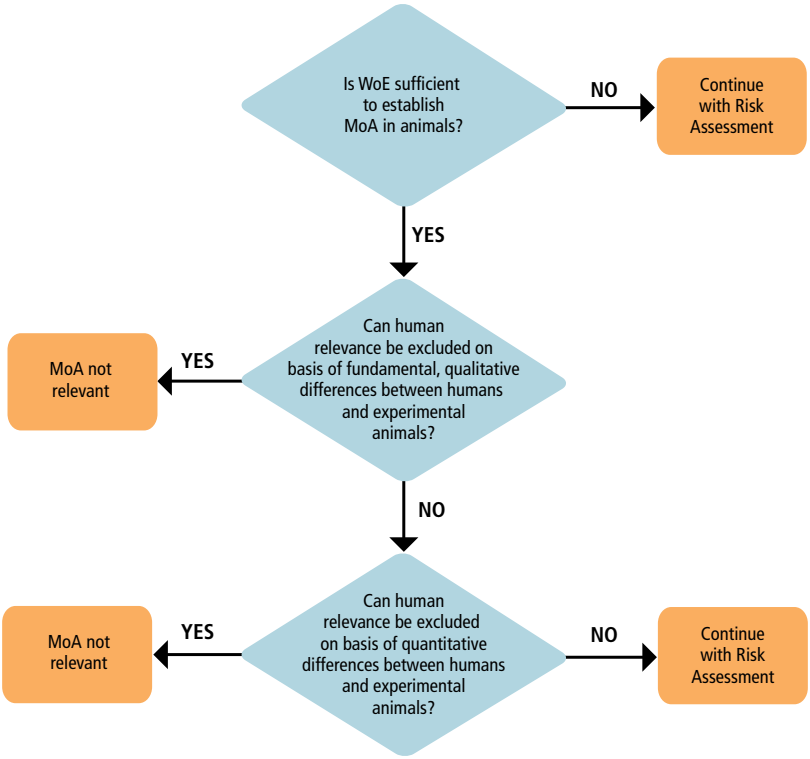
Building on earlier work that provided a framework to evaluate the MoA for chemical carcinogenesis (Boobis *et al*., 2006; Sonich-Mullin *et al*., 2001), the IPCS recently released a framework applicable to non-cancer endpoints (Figure 3.4) (Boobis *et al*., 2008). The central focus of the framework is on the hypothesized MoA, which comprises the "key events" causally related to the toxic effect, identified using an approach similar to the Bradford Hill criteria (Hill, 1965).

The first step in this framework is to establish a MoA in the experimental animal. This MoA describes the sequence of events that results in the observed toxicological outcome.

Once the MoA has been evaluated in an experimental system, attention is placed on its human relevance. Four key questions are addressed:

1. Is there sufficient evidence to conclude that a MoA has been established in animals?
2. Are there fundamental differences between the experimental system and humans such that the key events are qualitatively not likely to occur in humans?
3. What is the evidence for quantitative differences in either kinetics or dynamics that would indicate a differential human sensitivity (presuming that the key events are qualitatively plausible in humans)?
4. To what extent do any quantitative differences in the key events impact the selection of dose-response approaches and uncertainty factors?

According to the IPCS, the utility of the human relevance framework extends beyond determining the human relevance of an animal-derived MoA by providing information that is useful in risk characterization. For example, it could point to modulating factors due to gender, life stage, age, or genetics, and the dose range over which the effects are likely to be induced. This latter point emphasizes the need for effective exposure assessments in developing risk management options. A number of case studies have been published using this approach (Klaunig *et al*., 2003; Meek *et al*., 2003; Seed *et al*., 2005). Furthermore, an umbrella plan for future international work to update WHO's MoA framework guidance was recently developed under the auspices of the WHO/IPCS Harmonization Project (WHO, 2011).



(Reproduced with permisson from *Critical Reviews in Toxicology*)*

Figure 3.4

Decision tree for determining human relevance of a MoA for toxicity observed in experimental animals

A number of case studies using this approach can also be found elsewhere (Meek *et al.*, 2003; Seed *et al.*, 2005).

*Reproduced with permission from: IPCS framework for analyzing the relevance of a noncancer mode of action for humans, Boobis, A. R., Doe, J. E., Heinrich-Hirsch, B., Meek, M. E., Munn, S., Ruchirawat, M. *et al.*, 38, 2008; permission conveyed through Copyright Clearance Center, Inc.

3.2.3 Building a Better Understanding of Biological Responses: Systems Biology and Computational Biology

In order to understand the key mechanistic responses that underpin a toxicological outcome and thus build AOPs and MoAs, it is necessary to comprehend how an organism functions at different levels of biological complexity and how these

levels of complexity interact with one another. At the heart of this are several research areas:

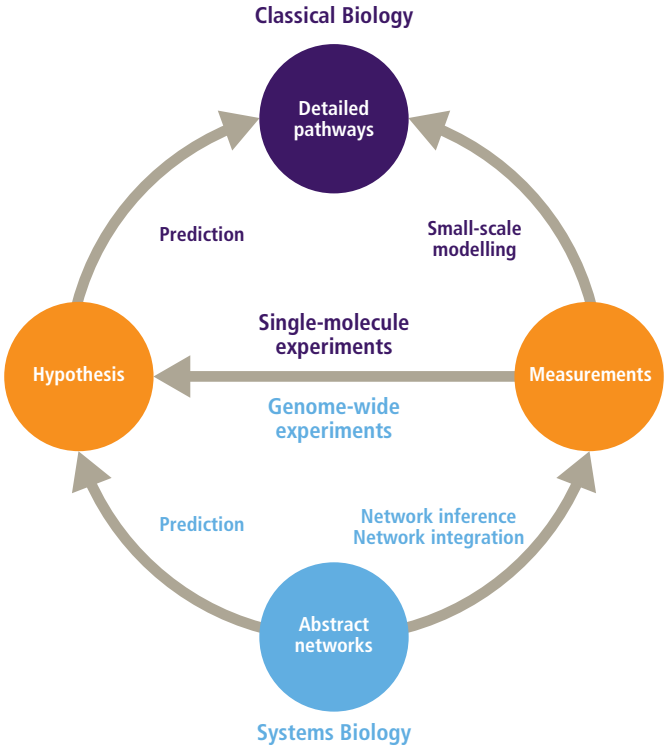
- pharmacology, which studies the effects of drugs (or other chemical compounds) on living systems;
- physiology, which studies the functions and activities of organs, organ systems, and whole organisms;
- pathophysiology, which studies the effect of perturbations on organs, organ systems, and whole organisms;
- systems biology, which seeks to identify and understand the implications of molecular and signalling interactions that take place within cells, and how these interactions result in the functions and behaviours exhibited by biological systems; and
- computational biology, which applies advances in computer science, applied mathematics, and statistics to the study of biological systems.

Particularly relevant to toxicology are the fields of systems biology and computational biology. These research areas seek to understand how normal systems-level functions can be perturbed after exposure to a chemical in order to develop computational tools to model and predict toxicological outcomes.

Systems Biology:

Advances in molecular biology have led to an increasingly reductionist approach to biological science, as the methods to study tissues, cells, and biomolecules such as proteins, RNA, and DNA have grown more sensitive and scalable. While this approach has led to numerous important discoveries, including the sequencing of the human and other genomes, it lacks the capacity to discover higher order (emergent) properties that represent the functional integration of the sub-components. The field of systems biology has emerged over the past decade to address this limitation.

Systems biology is the iterative and integrated study of biological systems across all levels of biological complexity, from molecular to organismal. It can be thought of as a framework for using genome-scale experiments to perform predictive, hypothesis-driven science (Figure 3.5). A key principle of systems biology is that it is not enough to simply map out the physical components and interactions of a system; it is important to know how information moves through the system in response to perturbations to appreciate both the normal and perturbed situations. Thus, by necessity, systems biologists must integrate multiple data from divergent sources in order to understand the system's performance.



(Reproduced with permission from *Annual Review of Cell and Developmental Biology*)*

Figure 3.5

Overview of the experimental process in classical biology (top) versus systems biology (bottom)

*Reproduced with permission from: A Decade of Systems Biology, Chuang, H. Y., Hofree, M., & Ideker, T., vol.26, p.721–744, 2010; permission conveyed through Copyright Clearance Center, Inc.

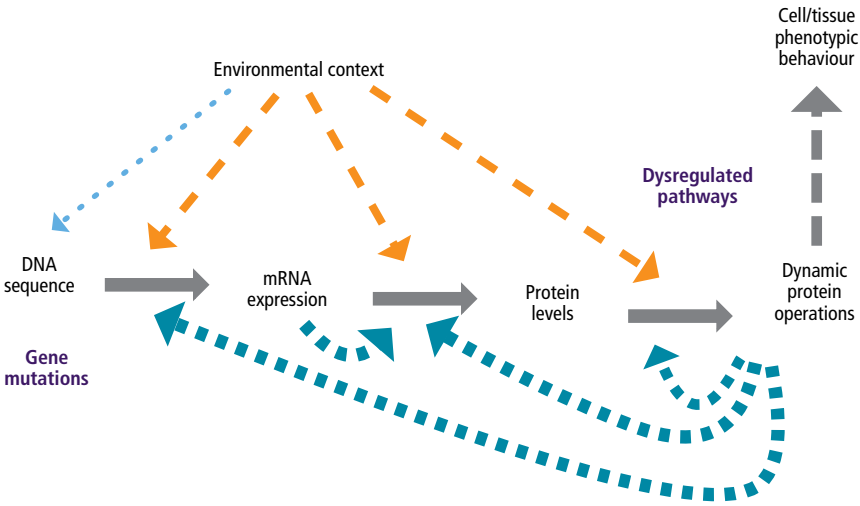
A recent review of progress in the field of systems biology described the rapid rate of development, but also highlighted that the continual introduction of new technologies and approaches has generated data faster than they can be assimilated (Chuang *et al.*, 2010). Chuang *et al.* (2010) also reviewed the content of publications on systems biology from 2001 to 2009. Some topics, such as gene expression analysis and evolutionary biology, have remained constant, while others, such as cancer research, stem cells, and network biology, have increased. This trend likely reflects the growing interest in the tools of systems biology to address those aspects

of living organisms that are more complicated. Using the example of molecular diagnostics, Chuang *et al.* (2010) showed how using systems-wide maps of cellular pathways could integrate seemingly divergent information arising from cellular and genetic heterogeneity. The outcome of such analyses does not identify individual genes or proteins as markers, but rather functionally related groups of genes or proteins (diagnostic pathway markers) whose overall expression is representative of phenotypic responses. In another example, pathway analysis was used to explore the factors controlling cell fate decisions in stem cells; here, expression levels of a network of 15 transcription factors (out of approximately 1,200) were strongly associated with cell fate. Further research is needed to understand how different internal and external stimuli can alter the state of the regulatory network and hence modify cell fate decisions, but this type of information will surely be critical to guiding efforts in tissue engineering and regenerative medicine. Ultimately, the Panel anticipates that understanding chemically-induced disease states may be of tremendous benefit to the field of toxicology.

Kreeger and Lauffenburger (2010) examined the challenges of understanding the results of genomic sequencing and profiling of transcripts, proteins, and metabolites of tumour cells with respect to therapeutic interventions, which has obvious linkages to toxicological interventions. Studies to date have shown that the molecular phenotype of tumours tend to contain heterogeneous modifications in dozens of different genes (TCGA, 2008; Jones *et al.*, 2008; Wood *et al.*, 2007). These authors came to the same conclusions as Chuang *et al.* (2010), namely that the need for and advantages of system-level approaches should be emphasized.

The notion that a group of key cellular pathways may be pathologically altered as a result of underlying genetic defects is an emerging organizing principle (Kreeger & Lauffenburger, 2010). The implications for drug discovery — and, by extension, toxicology — are that there are multiple targets within these pathways that can be examined for efficacy. Nonetheless, the complexity of signalling pathways, and their interactions, creates barriers to fully understanding cancer biology. As a consequence, it is likely that single diagnostic markers will not be very informative, and that elucidating a mechanistic understanding of pathway perturbations is unlikely without computational analysis. Importantly, the phosphorylation state of proteins must be a central measurement of pathway activity (Kreeger & Lauffenburger, 2010). Transcription and translation activity alone will not be enough to build an understanding of the dynamics of the system (Figure 3.6); rather, interactomes of protein-protein and protein-DNA relationships will provide the framework to advance this understanding. This is evidenced by the discovery of altered interactomes associated with several tumour types as well as the cellular

network changes for three pathways that are commonly mutated in cancers: p53, ErbB family of receptors, and RAS. In this example, systems biology seeks to elucidate the relationship between network alterations and key cancer processes at the cellular level (e.g., excessive proliferation, resistance to angiogenesis, and metastasis). These multi-scale system models represent a key direction in understanding normal and abnormal biology. Sloot and Hoekstra (2009) reviewed the research on a number of other multi-scale models focusing on cardiac, pulmonary, and musculo-skeletal systems within the International Union of Physiological Sciences Physiome Project.[66]



(Reproduced with permission from Kreeger & Lauffenburger, 2010)

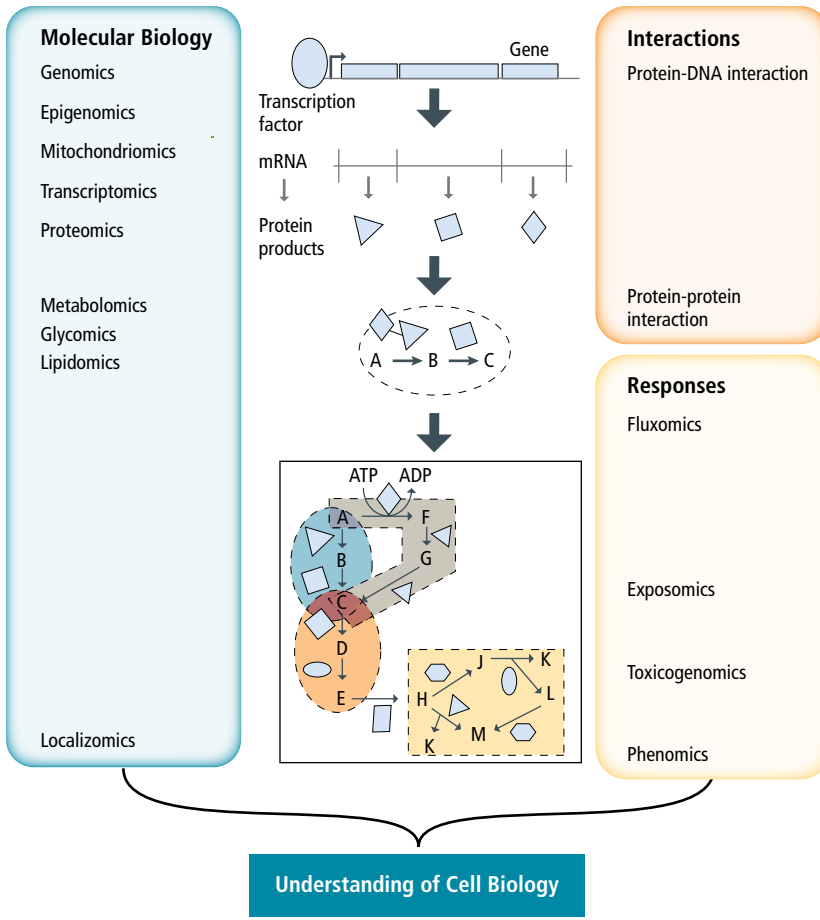
Figure 3.6
Schematic illustration of the molecular processes governing cell and tissue functional behaviour

Developing the systems-level understanding of the physiology needed to identify an interactome necessitates identifying and comprehending the constituent biological parts of the system. Recent advances in systems biology have arguably been fuelled by advances in both omics technologies and computational biology, which have helped systematically assimilate data sets that describe the molecular state of the cell.

66 The International Union of Physiological Sciences Physiome Project: http://www.physiome.org

Omics and Functional Genomics:

Omics describes a broad interdisciplinary endeavour that seeks to analyze the interactions of biological parts (or "omes"). Omics incorporates a multitude of diverse disciplines that map information from these constituent parts; identify relationships; and engineer them to understand and manipulate their regulatory mechanisms (Figure 3.7). Although it is only one of the components of the field, functional genomics has arguably been one of the most influential in driving advances in systems biology over the past 15 years.



(Reproduced with permission from Macmillan Publishers Ltd: *Nature Reviews Molecular Cell Biology*; Adapted and reproduced with permission from Joyce & Palsson, 2006)

Figure 3.7

Functional genomics seeks to develop a "systems-level" understanding at the level of an individual cell

Functional genomics seeks to relate genomic sequence data to biological outcomes by using the information obtained from genomes to assess gene functions and products and the translation of those products into larger macromolecules (Hieter & Boguski, 1997). As a discipline, it has advanced significantly since the release of the draft human genome sequence in 2001 (Lander *et al.*, 2001; Pevsner, 2009; Venter *et al.*, 2001).

Functional genomics encompasses a variety of technologies and techniques (e.g., microarrays, serial analysis of gene expression, genetic interaction mapping). It is largely focused on the macromolecular level (Pevsner, 2009). Revolutionary technologies generate vast amounts of data, thereby increasing the overall efficiency of the previously used "gene-by-gene" approach. Analyses and interpretations are now conducted using computational tools that were unavailable 15 years ago. Computational biology and bioinformatics tools are helping researchers develop a more integrated, systems-level understanding. These new data and technologies are being used in areas such as oncology, neurological diseases, disease susceptibilities, drug responses, and personalized medical interventions to further the capacity of biomedicine.

Human genome research has advanced, and the genomes of numerous other species have also been mapped.[67] These provide both the capacity for comparative genomics and a greater understanding of the organization and development of ecosystems. Advances in omics research can be used in the area of toxicity testing to provide important information about toxicologically relevant pathways and the effect of perturbations resulting from environmental exposure to specific agents (NRC, 2007). High-throughput, cell-based assays permit a functional analysis of how inhibiting or promoting gene expression can alter the activity of a toxicity pathway.

Computational Biology, Bioinformatics, and Chemoinformatics:
Computational tools provide the platform for sorting and collating omics data and making them available in a searchable and hierarchical form. Computational biology applies computer science, applied mathematics, and statistics to the study of biology. This field may be described as having two distinct branches (Kitano, 2002a):

- knowledge discovery, which includes data mining and the elucidation of patterns from experimental data. This approach is used widely in bioinformatics; and
- simulation-based analyses, which uses *in silico* approaches to develop predictions that can be tested *in vitro* and *in vivo*. This approach is directly relevant to IATA.

67 National Center for Biotechnology Information: http://www.ncbi.nlm.nih.gov/sites/genome

Bioinformatics describes the integration of biological measurements, computer science, and information technology to efficiently collate and analyze the large amount of data emanating from the growth of molecular biology and related disciplines (NCBI, 2003). It requires the development of computational tools that work on biological data in order to answer questions and solve problems (NIH, 2000). While the origins of bioinformatics may be traced back to the 1960s (Hagen, 2000; Searls, 2010), its major impetus was the processing of sequence data generated from the Human Genome Project. The approach quickly spread to the analysis of other omics data, including proteomics and transcriptomics.

The field of chemoinformatics (also known as computational chemistry) is analogous to bioinformatics but considers broad analyses of chemical compounds, their physical properties, and biological activities. Chemoinformatics has grown enormously in the last decade with the advent of public sector efforts in the U.S. (Austin *et al.*, 2004) and the EU (Hardy *et al.*, 2010).

The sheer volume of sequencing and chemical activity information has resulted in the development of many new algorithms and statistical approaches as well as methods to annotate and share information across various platforms and the creation of new visualization tools. Key factors advancing the fields of bioinformatics and chemoinformatics include the adoption of common ontologies (formal representations of a set of concepts within a discipline that capture relationships between them) (Box 3.5) and ongoing commitments to free and open access to the public.[68]

A systems-level approach necessitates a change in what we look for in biology (Kitano, 2002b). Although identifying all of the genes and proteins in an organism is a crucial step, it is but one step. Understanding how these pieces fit together to produce and control the complete organism is arguably the big challenge for systems biology. This level of understanding would ultimately permit the development of comprehensive models that can predict how organisms interact with and react to their environment (Latterich, 2005).

68 A short history of bioinformatics is available at http://www.netsci.org/Science/Bioinform/feature06.html

Box 3.5
Towards a Common Ontology for Toxicology

Efforts such as the Gene Ontology project helped to consolidate information from multiple sources by providing a controlled vocabulary — ontology — for describing gene product characteristics such as a cellular compartment, molecular function, and biological processes.[69] Relevant to toxicology are initiatives such as ToxML and OpenTox.

ToxML is a toxicology-specific tool that has been used to develop ontologies to standardize toxicology databases (Richard *et al*., 2008; Yang *et al*., 2006).[70] ToxML is designed to:

- Support broadly encompassing and meaningful representations of toxicology experiments with hierarchical schema that cover different levels of biological complexity; and
- Index data with chemical structures to ensure a database is useful for the widest range of biological studies.

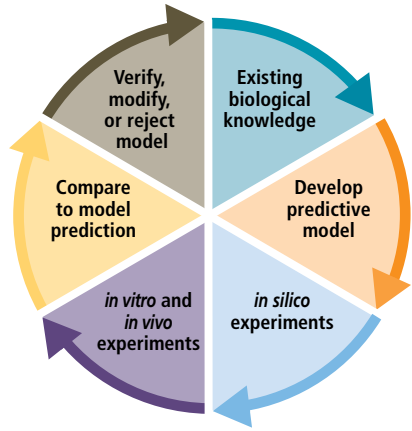
The OpenTox Database project is developing a toxicological endpoints ontology to aid organization of data and facilitate vertical and horizontal retrievals (i.e., within a given endpoint and between different endpoints) (Benigni *et al*., 2009).[71] The OpenTox ontology seeks to integrate the ToxML scheme with the data portfolios submitted in support of a chemical registration (e.g., the standard data sets discussed in Chapter 2) to make optimal use of all existing data.

The challenge of developing toxicological ontologies and data models, particularly for chronic mammalian endpoints, cannot be underestimated. There are a number of additional initiatives that seek to build on the OpenTox project and address this issue (e.g., the European Union's eTox Project and COSMOS).[72; 73]

Computational biology and chemistry are expected to play significant roles in the development of systems-level models, but this development will require an integrated and iterative process (Figure 3.8). This iterative process will be hypothesis-driven, with the development of progressively more accurate models based on

69 The Gene Ontology project: http://www.geneontology.org/
70 ToxML: http://www.leadscope.com/toxml.php
71 OpenTox: http://www.opentox.org/home/about
72 eTox: http://www.etoxproject.eu/
73 COSMOS Project: http://www.eclipse.org/cosmos/

increasing biological and chemical data. Models will be created *in silico* based on the available data, tested experimentally, and revised to address limitations. Importantly, predictions made by the computational models are iteratively tested using *in vitro* and *in vivo* experiments (Kitano, 2002b).



(Adapted and reproduced with permission from AAAS and Kitano, 2002b)

Figure 3.8

Building a systems-level model from component parts is an iterative process that uses existing knowledge in an integrated fashion

Relevant to toxicology is a systems-level understanding to identify those cellular perturbations that can lead to adverse health outcomes. This is the focus of the emerging field of computational toxicology. Computational toxicology adapts the tools of systems biology and computational biology to assess the risks posed by chemicals to human and environmental health.

3.2.4 Computational Toxicology

The US EPA defines computational toxicology as the "integration of modern computing and information technology with molecular biology to improve prioritization of data requirements and risk assessments" (US EPA, 2003a). It is a growing scientific field (Figure 3.9) that seeks to expand beyond earlier structure-activity modelling — which attempted to blend advances from modern computer science with those of chemistry and molecular biology — to improve the management of chemical exposures, hazards, and risks (Kavlock *et al.*, 2008;

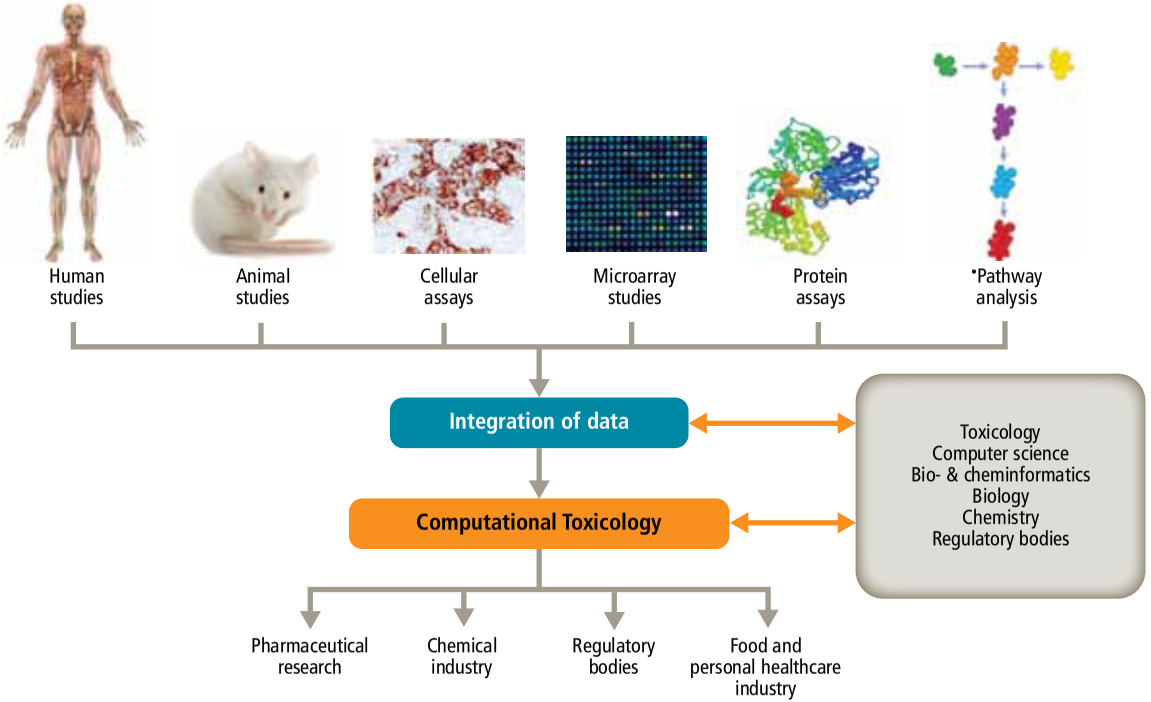


Figure 3.9

An overview of data sources and modelling methods used in computational toxicology

Kavlock *et al.*, 2009; Kavlock & Dix, 2010; Nigsch *et al.*, 2009). Ultimately, computational toxicology seeks to facilitate the development of simulation-based analyses to develop predictions that can be tested *in vivo* or *in vitro*. This, in turn, is directly relevant to elucidating MoAs and AOPs and advancing an IATA strategy.

Besides using reiterative computational modelling and experimentation, a major difference between current computational toxicology approaches and traditional toxicology is one of scale. Recent technological advances have significantly increased the breadth of endpoints and pathways that can be covered; the levels of biological organization that can be examined; the range of exposure conditions that can be considered; and the range of life stages, gender, and species factors that can be addressed (Kavlock *et al.*, 2008). The technological advances that have made this possible include the construction and curation of large-scale data repositories; the introduction of virtual- and laboratory-based high-throughput and high-content screening assays; and the introduction of computational models that can integrate information across sources and levels of biological organization (Kavlock *et al.*, 2008). (Much of the work to date has relied on existing models but there is still a lack of cell models for relevant endpoints. Considerable work is currently underway to develop new models; some of these initiatives will be discussed in more detail in Chapter 4).

A key component of computational toxicology is the development, population, and curation of chemoinformatics databases (Richard *et al.*, 2008). These databases must be established on standardized schema; developed in conjunction with subject matter experts for specific areas of toxicology; and populated with extensive data sets because data extractions are absolutely essential to support the maturation of predictive toxicology. The effective capture and representation of legacy data in a number of recent studies (Knudsen *et al.*, 2009; Martin, Judson, *et al.*, 2009; Martin, Mendez, *et al.*, 2009) illustrate the utility of building this chemoinformatics infrastructure.

Integrated and Interactive Knowledgebases:

Combining toxicity test data with physicochemical information creates the opportunity to develop SARs that should be more robust in generating predictions because they incorporate aspects of both chemical and biological space (Dix *et al.*, 2007; Houck & Kavlock, 2008). Predictive tools are only as good as the data

sets on which they are built. Computational toxicology requires that all existing toxicity data be compiled and organized in standardized and computable forms (R. S. Judson, 2010).[74]

Carefully curated information is needed in order to provide downloadable, structure-searchable, standardized files to ensure that structural analogues can be identified and that divergent data sets, such as those being generated by alternative testing methods, may be captured. As a result, the field is moving away from the use of linear databases and towards the development of relational databases and integrated knowledgebases.

Relational databases permit the storage, organization, mining, and sharing of data and metadata. Data are entered into a relational database by manually extracting them from existing (usually written) sources, including laboratory reports. An example of a scheme for a relational database is shown in Figure 3.10.

A knowledgebase is a database in which the data are organized in terms of ontologies (Box 3.5) that permit automated knowledge extraction from the data, including data residing in the open literature (R. S. Judson, 2010).[75] The organization of data within a knowledgebase can be quite different from that within a relational database. The scheme illustrated in Figure 3.11 uses only three tables:

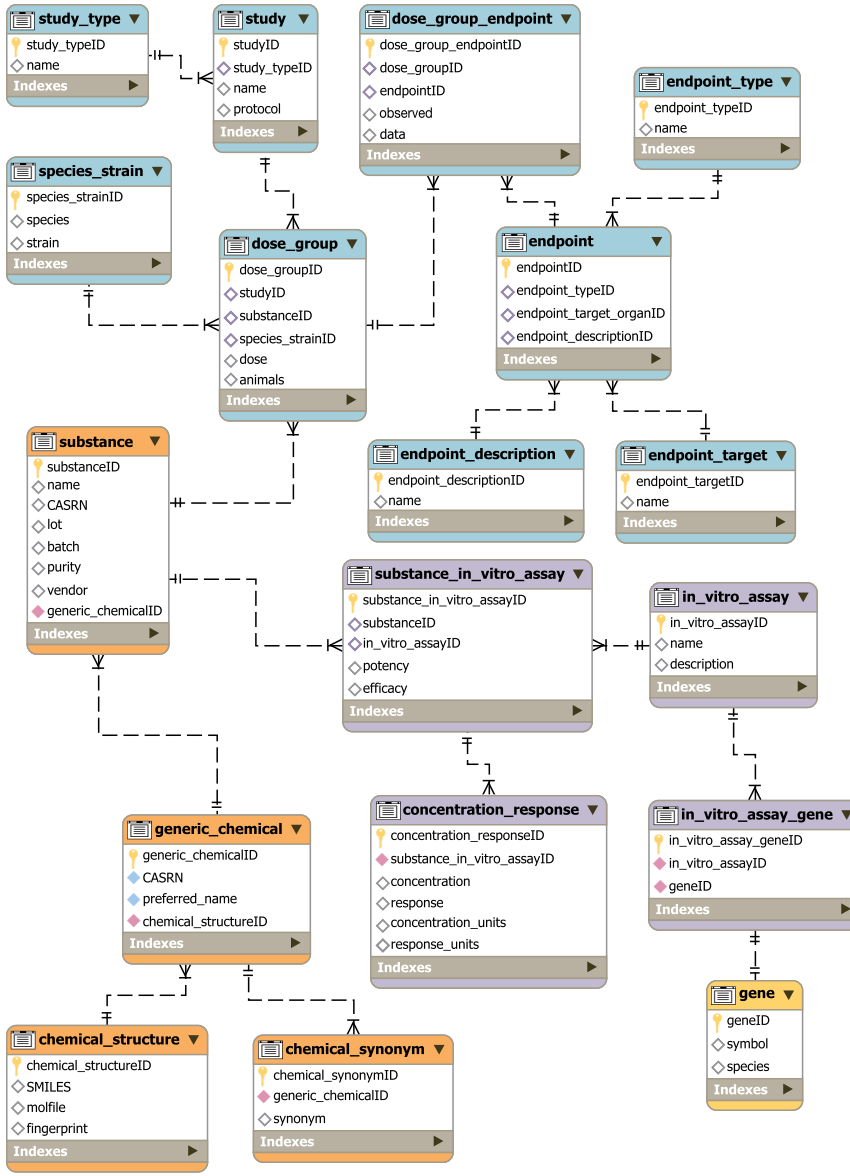
- Objects: Toxicity endpoint, chemical, and synonyms;
- Relationships between objects: Chemical causes certain toxic response; and
- Qualifiers for these relationships: Toxic response observed in specific species and at specific dose.

There are a number of online knowledgebases and databases (summarized in Table 3.2). Of these, the broadest database is the US EPA's Aggregated Computational Toxicology Resource (ACToR) (R. S. Judson *et al.*, 2008), which is a collection of over 500 public sources of information on over half a million chemicals.[76] ACToR is essentially a "database of databases," and its data sources include chemical structure, physical-chemical values, and *in vitro* and *in vivo* assay results.

74 The term "computable form" refers to databases in which the data are tabulated in a searchable way. This is in contrast to databases in which the information resides in text reports that are intended to be read (R. S. Judson, 2010).

75 For an example, see: http://www.gopubmed.org

76 ACToR: http://actor.epa.gov

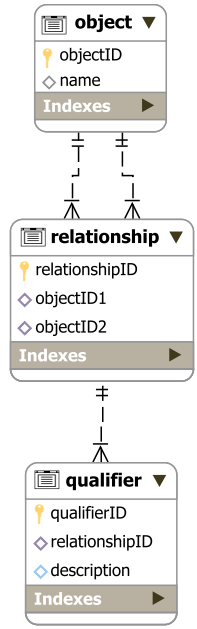


(Reproduced with permission from Taylor & Francis Group)*

Figure 3.10

An example of a relational database scheme designed to capture *in vivo* and *in vitro* data on test chemicals

*Public Databases Supporting Computational Toxicology, Richard Judson, *Journal of Toxicology and Environmental Health, Part B: Critical Reviews*, 2010, Taylor & Francis, reproduced with permission of the publisher (Taylor & Francis Group, http://www.informaworld.com).



(Reproduced with permission from Taylor & Francis Group)*

Figure 3.11
A simple database scheme designed to hold information in a knowledgebase

*Public Databases Supporting Computational Toxicology, Richard Judson, *Journal of Toxicology and Environmental Health, Part B: Critical Reviews*, 2010, Taylor & Francis, reproduced with permission of the publisher (Taylor & Francis Group, http://www.informaworld.com).

Included within the ACToR system is ToxRefDB,[77] which contains information extracted from regulatory toxicology studies covering developmental, reproduction, systemic toxicity, and cancer endpoints. ToxRefDB contains detailed study designs, dosing, observed treatments, and related effects using a controlled vocabulary. The database currently includes information on 474 chemicals, mostly pesticidal actives. This information is searchable by chemical, species, gender, and endpoint (Knudsen *et al.*, 2009; Martin, Judson *et al.*, 2009; Martin, Mendez *et al.*, 2009). Also included in the ACToR system is the US EPA's Distributed Structure-Searchable-Toxicity Database (DSSTox), which was one of the first attempts to provide the public with curated information about chemicals and their responses in a number of biological test systems.[78] Currently well recognized, it is used by researchers exploring SARs (Box 3.6) (Richard & Williams, 2002; Richard *et al.*, 2008).

Table 3.2

Summary of relevant databases and knowledgebases

Data Source	Tabular/Computable	Searchable by Structure?	Type
Online databases of *in vitro* and *in vivo* data			
ACToR*	Some data are tabular	Yes	Database of databases
OECD eChemPortal	Some data are tabular	Yes	Database of databases
Online databases of *in vivo* data			
CPDB	Yes	Yes (via DSSTox)	Database
DSSTox	Yes	Yes	Database
DrugBank	Yes	No	Database
EPA ECOTOX	Yes	No	Database
HPVIS	Yes	No	Database
NTP	No (but future version will be)	Yes (via DSSTox)	Database
TOXNET	Yes	No	Database portal
ToxRefDB	Yes	Yes (via DSSTox)	Database
Online databases of *in vitro* data			
CEBS	Yes	Yes (via DSSTox)	Knowledgebase
PubChem	Yes	Yes	Database
CTD	Yes	No	Knowledgebase
Online toxicology knowledgebases and ontologies			
OBO Foundry	Not applicable	Not applicable	Ontology compilation
GO3R	Yes	Not applicable	Knowledgebase
GoPubMed	Yes	Not applicable	Knowledgebase

(R.S. Judson, 2010)

*ToxCAST™ data included in ACToR in January 2011.

77 ToxRefDB: http://www.epa.gov/ncct/toxrefdb/

78 DSSTox: http://www.epa.gov/NCCT/dsstox/index.html

Box 3.6
CASE STUDY: ACToR and Data Management

Data sources included in ACToR must be publicly available, contain information on chemicals of interest to the US EPA, be indexed by chemical (i.e., there must be data on individual chemicals), and be indexed by the Chemical Abstracts Service (although there are some exceptions to this).

The ACToR system was used to survey *in vivo* mammalian toxicity data on over 10,000 chemicals. This list of chemicals included HPV chemicals; medium production volume chemicals; pesticidal and antimicrobial agents and formulants; known drinking water contaminants; hazardous air pollutants; and certain classes of defined chemicals including the US EPA Toxic Release Inventory, the Integrated Risk Information System (IRIS), and the Endocrine Disruptor Screening Program (EDSP) candidate list (R. S. Judson *et al*., 2009). Of the 10,000 chemicals queried 34.0 per cent had no available toxicity information, 58.6 per cent had acute toxicity data, and 10.8 per cent had reproductive toxicity data.

Summary of overlap between the 9,912 chemicals queried under ACToR and the set of accompanying assay components.
Percentages are shown in parentheses.

Assay	Tabular *in vivo* Data	Non-tabular *in vivo* Data	Summary Data from Risk Assessment	Summary Data from Text Reports Online	Data from any Source
Hazard	4,454 (44.9)	0	255 (2.6)	4,767 (48.1)	5,810 (58.6)
Carcinogenicity	1,211 (12.2)	401 (4.0)	726 (7.3)	2,035 (23.3)	2,579 (26.0)
Genotoxicity	2,496 (25.2)	1,102 (11.1)	32 (0.3)	1,047 (10.6)	2,724 (27.5)
Developmental toxicity	755 (7.6)	37 (0.4)	125 (1.3)	2,324 (23.4)	2,862 (28.9)
Reproductive toxicity	734 (7.4)	0	31 (0.3)	396 (4.0)	1,081 (10.9)
Food safety	1,692 (17.1)	0	533 (5.4)	0	2,258 (22.8)

(R.S. Judson *et al.*, 2009)

The power of these kinds of large databases lies in the ease and speed with which numerous chemicals can be queried; a thorough manual search for information on 10,000 chemicals would have been almost impossible. The use of such tools may facilitate the rapid screening and prioritization of large numbers of chemicals.

continued on next page

Box 3.6 *(continued)*

These chemicals may subsequently be candidates for programs such as ToxCast™ that require traditional toxicity data to develop predictive models. The outcome also points to the dire need for more efficient and effective toxicity testing approaches that can close the huge public information gap on the hazards of chemicals.

These kinds of initiatives are critical to advancing the capacity of computational toxicology to develop predictive models for human and ecosystem toxicity. Considerable work has been done to enter data into relational databases; however, there remains an enormous need to digitize existing toxicity data in order to ensure that models are developed on the best and most complete data sets available. Furthermore, not all published or existing data are of equal quality; therefore, existing data should be considered part of a weight-of-evidence (WoE) approach.

3.3 THE STATE OF THE SCIENCE OF ALTERNATIVE TESTING TOOLS AND DATA SOURCES

Computational toxicology was once defined primarily as the application of structure-activity models to predict the effects of chemicals based upon their structural similarity to chemicals whose effect is known. As described above, as computational capacity has increased dramatically in recent years, so too has the scope of computational toxicology. Although structure-activity models are expected to remain at the heart of computational toxicology, they will be improved upon by including knowledge and understanding derived from systems biology. The following section highlights some of the emerging tools that could help to translate advances in the knowledge of underlying biology into advances in the modelling of toxicity outcomes.

3.3.1 The Threshold of Toxicological Concern

The threshold of toxicological concern (TTC) describes a level of exposure that represents negligible risk. It can be used as a surrogate for safety data in the absence of chemical-specific primary toxicity data (Munro *et al.*, 2008). It is widely acknowledged to be a useful structure-activity relationship (SAR)-based concept for use in a regulatory environment.

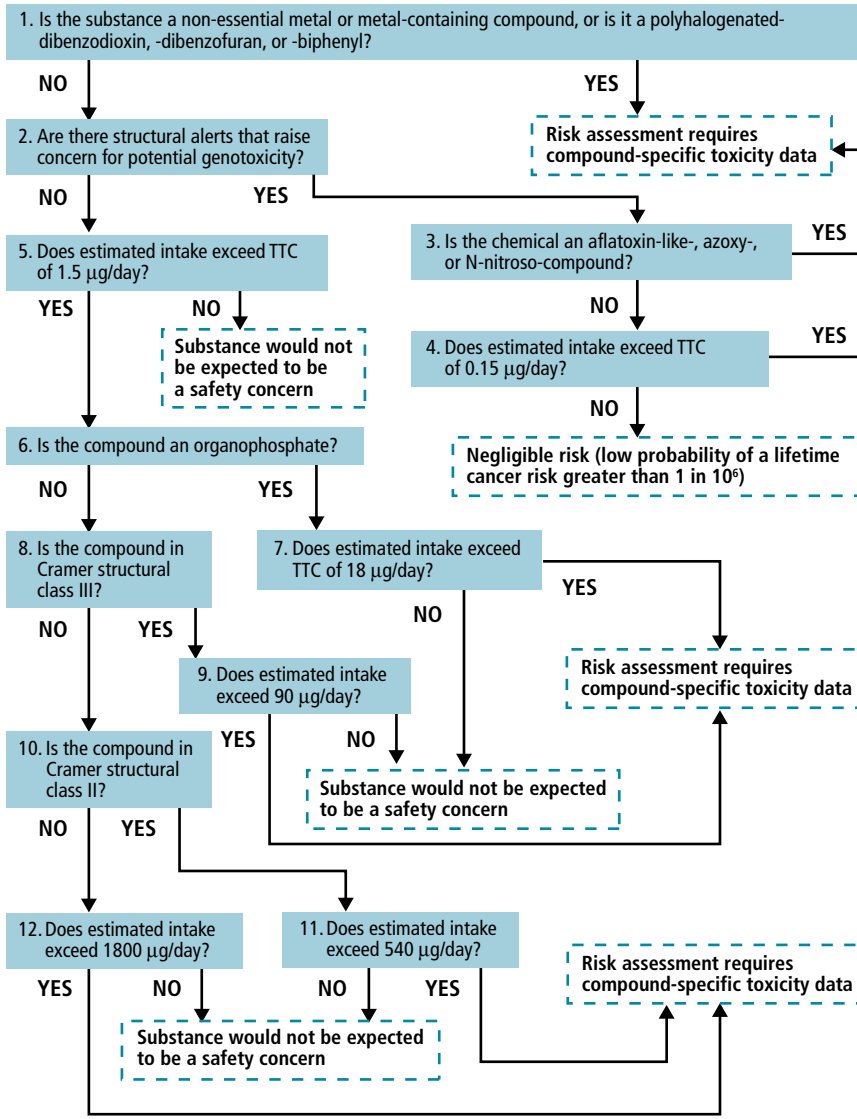
TTCs are applied to chemicals with predicted or known levels of exposure but limited toxicity data. They use data from structurally related chemicals of known toxicities for both carcinogenicity and systemic toxicity endpoints (i.e., conservative values derived from distributions of no observed effect levels).

The TTC concept was initially proposed by Frawley (1967) for substances intended for use in food-packaging materials. It was extended by Munro *et al.* (1996) who developed human exposure thresholds for each of three structural classes of chemicals based on the Cramer decision tree. The Cramer decision tree uses a series of questions to seek, sort, and classify chemicals into one of the three classes (Cramer *et al.*, 1978):

- Class I: Simple-structure chemicals that are efficiently metabolized and have a low potential for toxicity.
- Class II: Chemicals of intermediate concern that are less innocuous than class I substances but that lack the positive indicators of toxicity that are characteristic of class III chemicals.
- Class III: Chemicals with structures that suggest significant toxicity or for which it is not possible to presume safety.

Human exposure thresholds were developed for chemicals in each of the three structural classes using NOAEL data (in mg/kg of bodyweight per day) from chronic and subchronic rodent or rabbit studies. The NOAEL distributions were plotted, and human TTC values for each chemical class were derived by dividing the NOAEL value from the 5th percentile by a 100-fold uncertainty factor and multiplying by 60 kilograms (Munro *et al.*, 1996). The derived values — 1,800, 540, and 90 micrograms per day for class I, II, and III, respectively — have since been incorporated into the risk assessment approach for food flavourings (Munro *et al.*, 2008).

Particularly relevant to IATA is the development of a TTC decision tree. Such a decision tree incorporates the aforementioned human exposure values and is used to determine whether further toxicity data are needed to inform a risk assessment. The first step in this tree (Figure 3.12) considers whether it is appropriate to apply the TTC concept and, if so, whether the existing data set for structurally similar chemicals is adequate for using the TTC. If it is, subsequent decision points in the tree are arranged in descending order of toxicological concern and potency, so compounds that cannot be addressed using a TTC approach are eliminated early (Kroes *et al.*, 2004).



(Adapted and reproduced with permission from Elsevier)*

Figure 3.12

Thresholds of toxicological concern decision tree

*Reproduced from: *Food and Chemical Toxicology*, 42/1, Kroes, R., Renwick, A. G., Cheeseman, M., Kleiner, J., Mangelsdorf, I., Piersma, A. *et al.*, Structure-based thresholds of toxicological concern (TTC): Guidance for application to substances present at low levels in the diet, 65-83, Copyright (2004), with permission from Elsevier.

The utility and predictivity of any comparative study are only as powerful as the data set on which it is built. Although the robustness of the TTC approach has been shown to be scientifically credible for addressing food additives (a subject that will be explored in more detail in Chapter 4), the structural definitions of the classes (developed by Cramer in 1978) have not been validated against more recent toxicity data or SARs (reviewed in Munro *et al.*, 1996). Today there are many ongoing initiatives to explore, expand, and improve the TTC approach, the outcomes of which may have broad applicability to IATA for chemicals in general and pesticides in particular (EFSA, 2009a; ILSI, 2005).

3.3.2 Structure-Activity Relationships and Chemical Categorization

Structure-activity relationships (SARs) look at how the structure of a molecule influences its behaviour within a system. The fundamental assumption in SAR studies is that molecules with similar structures will behave in similar ways. A number of factors affect the behaviour of a compound in biological or environmental systems, including its solubility, clearance rate, transportability, and the molecular entities to which it binds. Many of these features can be linked to the structure of the agent in question, which in turn is related to its various molecular characteristics.

Qualitative SARs derived from non-continuous data (e.g., yes-or-no data) and quantitative SARs derived from continuous data (e.g., toxic potency data) are collectively referred to as (Q)SARs.[79] The (Q)SAR method is not novel; Cros describes the relationship between the toxicity of primary aliphatic alcohols and their water solubility as early as 1863 (Cros, 1863). The structure-activity models used today grew out of the work of Corwin Hansch in the 1960s (Hansch & Leo, 1979). (Q)SAR is recognized internationally as an alternative to low-throughput toxicity testing, particularly in ecotoxicology, as reflected by a number of OECD initiatives and summarized in a series of reports (OECD, 2004e, 2006, 2007b, 2007c).

(Q)SAR methods have seen regulatory use for a number of years, particularly for ecotoxicity assessments and hazard identification for data-poor chemicals. As discussed in Chapter 2, the first step in hazard identification is determining the adequacy of existing data for each evaluated toxicity endpoint. If sufficient data are not available (which is often the case for industrial chemicals), more data are required to complete an assessment. One approach to filling these data gaps is to group chemicals into categories based on their physicochemical and toxicological properties (including common MoAs) and to use primary toxicity data on some category members to estimate missing values for untested members (OECD, 2009a; van Leeuwen *et al.*, 2009).

79 OECD: http://www.oecd.org/document/29/0,3746,en_2649_34379_42675741_1_1_1_1,00.html

A chemical category can be expressed in a chemical categorization matrix, which can cross-reference the category members against data describing chemical properties and toxicological endpoints (Box 3.7) (OECD, 2007d; van Leeuwen *et al.*, 2009). This can be used to evaluate all category members for common toxicological properties and to help identify data trends related to a specific endpoint. In turn, identifying common trends for chemicals within a category increases the confidence in the results, which increases their utility (and appropriateness) for use in hazard identification and characterization.

Box 3.7
CASE STUDY: Chemical Characterization and Filling Data Gaps

Structure-activity relationship studies are used to predict properties and toxicities of an untested chemical by drawing inferences from other members within its category. The table below shows a matrix that cross-references five hypothetical chemicals (A, B, C, D, and E) against structural, physicochemical, and toxicological data. This matrix indicates that chemicals A, B, D, and E form a single category because structure S1 and property P1 are common to all four and related to property P2 and toxicities T1 and T2. C is a separate category. Two data gaps for toxicity are observed:

- T1 for chemical E
- T2 for chemical D

The data in the Table below indicates that structure S1 and toxicity T1 correlate for the four compounds where data are available, suggesting that the T1 will be positive for compound E. Similarly, in the four available instances, the toxicity number T2 is five times that of property P2 plus five, which predicts a score of approximately 25 for compound D. This simple example illustrates the potential of SAR studies to fill knowledge gaps about chemical toxicity.

Structure/Property/Toxicity matrix for five chemicals within the same group

	Chemical A	Chemical B	Chemical C	Chemical D	Chemical E
Structure S1	+	+	–	+	+
Property P1	+	+	–	+	+
Property P2	1	2	3	4	5
Toxicity T1	+	+	–	+	?
Toxicity T2	10	15	20	?	30

The data from a chemical category matrix can be used in an IATA approach to address data gaps using either read-across or (Q)SAR modelling (OECD, 2009b).[80] Read-across is the simpler method that predicts endpoint information for one chemical by using data for the same endpoint from another similar chemical (or group of chemicals) (Schultz *et al.*, 2009).[81] (Q)SAR models are used when the category is of sufficient size and the OECD (Q)SAR validation principles are met (see OECD, 2007b for more information).

The predictive accuracy of read-across/(Q)SAR approaches is based on the assumption that the *a priori* binning of a chemical is correctly categorized. As a result, selection of the wrong chemical category represents a much greater potential source of error than the incorrect prediction of potency within the correct category. The utility of these approaches therefore depends on the existence of data on a large number of structurally characterized chemicals in order to permit accurate category formation.

Category Formation by Chemical Structure:

The three-dimensional structure of a chemical uniquely defines its attributes. As such, the structure of a chemical can be used to estimate its physical properties, biological activity, and environmental behaviour.

As discussed above, chemicals may initially be grouped based on their physicochemical properties (e.g., chemicals that react covalently with thiol groups would constitute a chemical group). Commonalities in structure between members of the group lead to the identification of structural alerts, which can be used to infer toxic properties based on chemical reactivity; chemicals that can react covalently with macromolecular structures to elicit toxicological effects may be subcategorized by the nature of their covalent reaction(s). Structural alerts can then be used to define the molecular structural limits of the domain and assign a chemical to an appropriate category based on its potential reactivity profile (Box 3.8).

80 The term "(Q)SAR" is used throughout this report to reflect the predictive nature of any relationships between chemical structure and biological activity without regard for it being qualitative or quantitative in character.

81 Read-across may be conducted in one of two ways, depending on the availability of primary data on other members of the chemical category (OECD, 2007d). If the number of closely related chemicals is large (greater than 10), the category approach is used; if the number is smaller than 10, the analogue approach is adopted. In the analogue approach, endpoint information for a small number of tested chemicals is used to predict the same endpoint for a similar (analogous), chemical. In the category approach, the endpoint data from several chemicals is used to predict the same endpoint for the similar, untested chemical.

Box 3.8
An Aside on Structural Alerts and Mechanistic Domains

There are believed to be seven "mechanistic" domains that define the nature of the covalent interaction between a chemical and the biological macromolecule that leads to a molecular initiating event (reviewed in Enoch & Cronin, 2010). These mechanistic domains are as follows:

- Michael addition
- acylation
- Schiff base formation
- aromatic nucleophilic substitution
- unimolecular aliphatic substitution
- bimolecular aliphatic nucleophilic substitution
- reactions involving free radicals

A review of the literature identified 57 unique structural alerts relevant to toxicity outcomes, each of which was assigned to a mechanistic domain (Enoch & Cronin, 2010). For example, the structural alerts for Michael additions consists in an unsaturated bond (i.e., an alkene or alkyne) with a neighbouring electron-withdrawing group (Schultz *et al*., 2007). These can include α,β-unsaturated carbonyls, quinones, quinomethanes, indoles, and heterocyclic rings (reviewed in Enoch & Cronin, 2010).

Members of a chemical category may share a common toxicological mechanism of action (OECD, 2007d); therefore, assigning a chemical to the correct category is of critical importance (Aptula & Roberts, 2006; Enoch *et al*., 2009; Enoch *et al*., 2008). Chemical categorization based on the likelihood of covalent adduct formation and other exclusion rules based on additional properties may be relevant for characterization and would permit the grouping of chemicals into toxicologically meaningful groups (whether a category or subcategory) for use in a regulatory context (Enoch & Cronin, 2010; Schultz *et al*., 2006).

A structural alert associated with Michael addition is defined by the presence of a polarized double or triple bond. Michael addition is believed to play a role in toxicity

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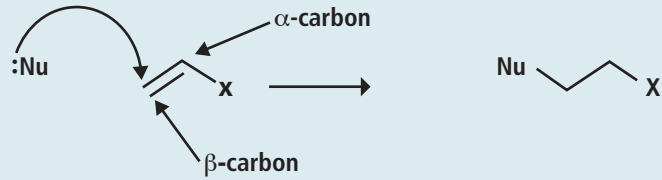
Box 3.8 *(continued)*

mechanisms relevant to skin sensitization and genotoxicity; chemicals that act as Michael acceptors react covalently with DNA and proteins (reviewed in Wondrousch *et al*., 2010).



(Reproduced with permission from *Critical Reviews in Toxicology*)*

Michael addition involves a nucleophilic attack on a polarized unsaturated compound

*Reproduced with permission from: A review of the electrophilic reaction chemistry involved in covalent DNA binding, Enoch, S. J., & Cronin, M. T. D., 40/8, 2010; permission conveyed through Copyright Clearance Center, Inc.

Although structural alerts can be very useful, they are not absolute predictors because the activities are context-dependent. Structural alerts increase the likelihood of a chemical effect, but conclusions about such activity can only be determined experimentally in a specific context. For example, Michael addition includes a number of subcategories where potency is consistent within the subcategory but varies between subcategories. The classic example is the difference in fish acute toxicity between acrylates and methacrylates, with the former being many times more toxic than the latter (Reinert, 1987). Such examples suggest that a more systematic approach to refining chemical categories or to subcategorization may be required to better incorporate potency differences into predictions, and thus assure a greater likelihood that the estimated value will be accurate.

Furthermore, biological processes are inherently chiral and involve interactions between molecules (e.g., proteins, enzymes, nucleic acids, membranes, etc.) whose three-dimensional structures are defined by energy relationships. The chirality of a chemical is therefore an important factor in determining its biological activity. This phenomenon is exemplified by comparing enantiomers — chemicals that are structurally almost identical but differ in their "handedness" (i.e., they possess one

or more chiral centres) — which can exhibit very different biological effects. Thus assigning a chemical to its appropriate chemical category necessitates consideration of its steric and physicochemical properties. It speaks to the need to make chemical categories, for the purposes of toxicity screening, toxicologically meaningful.

The Value of Toxicologically Meaningful Categories:

A toxicologically meaningful category is a group of chemicals whose toxicological profiles are likely to be similar or follow a regular pattern (reviewed in Bassan & Worth, 2008). This may permit a transparent, defensible assessment through mechanistic comparisons without further testing.[82] Using toxicologically meaningful categories shifts the emphasis towards intrinsic chemical activity and critical biological events and away from statistical parameters, especially a fixation on fit and predictivity.

Toxicologically meaningful categories are typically based on molecular similarity, common chemical reactivity, or shared modes of toxic action (OECD, 2009b); however, confidence in the assignment diminishes as one moves from a common chemistry-based mechanism to a biology-based mechanism.[83] The first reduction in confidence is due to the lack of biological mechanisms that have been completely delineated. The second reduction in confidence occurs since there is no best, accepted way to define molecular or structural similarity and even small changes in structure may result in large changes in behaviour and toxicity.[84]

Intuitively, the common chemical reaction seems to be a good way to classify a chemical. This is further supported by the literature (Enoch *et al.*, 2009; Enoch & Cronin, 2010; Swanson *et al.*, 1997). When the chemicals in a category exhibit a single mechanism of action, the categorization represents a powerful and pragmatic means by which the structural requirements of that mechanism may be described. The confidence in the category is significantly greater when the number of tested chemicals is greater and also when the members of the category share a common mechanism of action; however, our current knowledge of toxicological categories and category formation is limited. This is largely due to the lack of depth, breadth, and availability of data needed to support category formations, coupled with the complexity of the hazard endpoints being evaluated.

82 The term "toxicologically meaningful category" considers both the endpoint and the exposure scenario. For example, the same MoA might lead to acute mortality in both fish and mice; however, the toxicologically meaningful category for fish will be limited by water solubility while for mice it will be limited by vapour pressure.

83 This is even more so when molecular similarity is considered.

84 This also presumes that a chemical will have only biologically-based mechanisms of concern, which is probably not true.

One limitation in developing predictive capabilities with chronic endpoint data is that the underlying physiological response (i.e., the mechanism of toxicity) is largely ignored when the focus is on the hazard endpoint (e.g., Lowest Observed Effect Concentration [LOEC] or No Observed Effect Concentration [NOEC]). The result is that there is no basis to separate chemicals into biologically based categories. Clearly, structural alerts are a successful effort that introduces mechanistic knowledge into the category-based prediction.

The concept of toxicologically meaningful chemical categories and the ideas of read-across or (Q)SAR modelling for data filling are coupled so the general explanation of the category concept and the historical description of read-across and (Q)SAR modelling are equivalent. Many of the well-studied toxicity (Q)SARs have applicability domains that can be mechanistically derived from experimental data that have quantified critical events along the pathway (Bradbury *et al.*, 1990). Forming categories for endpoints is well established, especially when developed along the OECD validation principle of mechanistic plausibility (OECD, 2007b, 2009b). Categories based on chemical reactivity (or lack of it) are also well recognized.[85] Moreover, categories may be augmented by some information on mechanisms or modes of toxic action; however, to assign every discrete organic chemical to a category for each hazard endpoint of interest will necessitate the development of new toxicologically meaningful categories.[86]

When the molecular initiating event is closely linked to an *in vivo* response, a (Q)SAR model that relates the *in vivo* endpoint to the chemical structures may be derived; however, such direct linkages are typically not available for chronic effects and cannot be reliably predicted using such models. Moreover, without a transparent description of a plausible progression of adverse effects at the different levels of biological organization, it is difficult to reliably categorize chemicals based on similarity in toxicological behaviour.

As discussed earlier, confidence in an AOP increases with greater understanding of the interactions between the chemical and biological systems. The challenge therefore becomes one of forming toxicologically relevant categories that allow for structurally defined applicability domains for endpoints with intricate pathways based on clustering-like symptoms resulting from multiple events that accumulate

85 Especially electro (nucleo) philic interactions, where the applicability domains are based on conventional organic chemistry.

86 This is particularly relevant for complex endpoints that result from multiple events (e.g., repeat dose toxicity), multiple exposures that accumulate over time (e.g., neural toxicity), or are particular to a life stage of the organism (e.g., developmental toxicity).

over time or are particular to a certain life stage. Binding of the estrogen receptor (ER) provides an excellent example of how more than one structural domain is related to the same adverse outcome by a series of biological processes (Box 3.3) (OECD, 2009j). ER binding constitutes the molecular initiating event; the resultant adverse outcomes are complex and typically include reproductive and developmental effects. The ER has a "dynamic and plastic character" that is sufficiently non-specific to permit binding with a range of compounds (Katzenellenbogen *et al.*, 2003). This is in contrast to the lock-and-key nature of other hormone receptors that exhibit a higher degree of specificity in substrate-binding (Katzenellenbogen *et al.*, 2003). It is believed that the ER possesses three primary subpockets (typically referred to as sites A, B, and C), each of which has different hydrogen-bonding requirements (Tedesco *et al.*, 2001). The nature of the chemical interaction with the ER determines the subpocket to which binding occurs. This knowledge permits the formation of categories based on the molecular initiating event of subpocket binding. Altered gene expression from ER binding can be measured quantitatively using an engineered ER binding promoter sequence linked to a bioreporter system (Sanseverino *et al.*, 2009; Schultz *et al.*, 2002).

An AOP shifts the emphasis for category formations away from intrinsic chemical activity and towards the combined effects of chemical activity, plus the key events that occur across the different levels of biological organization. The AOP places chemicals into categories based on data that are more manageable than that required for delineating its "mechanism of action." Nevertheless, the categories are still toxicologically meaningful to fill data gaps in a transparent and mechanistically plausible manner.[87]

Furthermore, knowing the physicochemical properties of a chemical (or category of chemicals) and the AOP as well as understanding the toxicological MoA can be used to improve existing approaches to toxicity testing. For example, the Local Lymph Node Assay (LLNA), currently the accepted method of evaluating the skin sensitization potential of a chemical, demonstrates this. Both the fundamental chemical basis of protein binding and the chronology of the biological events leading to skin sensitization are sufficiently well-understood to elucidate a MoA. From this MoA, an AOP for skin sensitization has been derived. The events in the AOP represent excellent targets for developing *in vitro* alternatives to the LLNA (Box 3.9).

87 The AOP and MoA are similar conceptual frameworks that describe existing knowledge concerning the linkage between a series of key events and an adverse outcome at a biological level of organization relevant to risk assessment. The AOP conceptual framework originated in the ecological community interested in population effects, while MoA weight-of-evidence framework originated in the human health community looking at the human relevance of the lab animal results.

Skin sensitization happens via a T-lymphocyte-mediated immune response. An allergen penetrates the skin and forms a covalent complex with a carrier protein. This complex must be sufficiently antigenic to stimulate an allergic response that results in the production of memory and effector T-lymphocytes. Subsequent exposure to the chemical will then result in clinical allergic contact dermatitis.

Box 3.9
CASE STUDY: Using the MoA and AOP to Improve the Local Lymph Node Assay

The Local Lymph Node Assay (LLNA) measures the proliferative response of lymph node cells after topical exposure of a mouse to the test substance. Although the precise reaction mechanisms may vary depending on the chemical in question, the MoA for skin sensitization is well established and the AOP may be described as follows:

- Penetration into the viable epidermis (bioavailability);
- Formation of a stable and immunogenic protein-chemical complex (molecular initiating response);
- Induction of sufficient dermal trauma to induce an immune response by the epidermal Langerhans cells (cellular-level response); and
- Induction of T-lymphocyte response (organ-level response).

The molecular initiating event is believed to be the formation of a covalent complex between the chemical and a protein. It is generally agreed that any substance that covalently bonds to proteins has the potential to be a skin sensitizer (Gerberick *et al*., 2008). Thus, it is essential that any alternative strategy for assessing skin sensitization includes the means of selecting the most likely reaction as well as capturing relative reactivity, at least in the context of extreme/strong, moderate, weak, or nonreactive. In contrast to receptor-mediated chemical interactions (e.g., ER binding), electrophiles are not specific to their molecular targets. As a result, identifying the specific target protein is not critical to predicting skin sensitization; however, nucleophilic sites related to skin sensitization do vary, so knowledge of the reaction chemistry (and associated chemical space) is critical to using the AOP.

Another supplementary step in identifying protein binding is metabolism and abiotic transformation of a chemical. *In vivo*, the keratinocyte is the primary site of metabolism. While *in silico* methods for identifying reactive metabolites exist, their current predictivity varies depending on the reaction being simulated.

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Box 3.9 *(continued)*

The identities of the cellular pathways perturbed by the reactive chemicals have not been completely elucidated; however, there is evidence that mitogen-activated protein signalling pathways are critical to skin sensitization (see Vandebriel & van Loveren, 2010, for further discussion). A possible alternative for detecting reactive chemicals that act by covalent binding to thiol groups is Nrf2 oxidative stress. The Keap1-Nrf2-ARE assay is a luciferase reporter system (Natsch & Emter, 2008), which is a cellular pathway-based assay that is relevant to skin sensitization through its measured endpoint (Natsch, 2010).

In vitro assays for sensitization using cell or tissue cultures are particular to the cellular-level response. Typically, such assays have as their endpoint a single event associated with the stimulation of dendritic cells. Measurement of the expression of certain molecules or of secretion of specific cytokines have all been reported (reviewed in Vandebriel & van Loveren, 2010). However, no assays for different chemical reactions or the different thiol and amino targets have as yet been robustly evaluated. It is unclear if dendritic cell recognitions/activations are themselves key events or components of a larger cascade of biological events that follow the molecular initiating event.

In vitro proliferation of naïve lymphocytes has also been proposed as an alternative method related to skin sensitization (Jowsey *et al*., 2006); however, it is unlikely that any alternative method representing an event this far along the AOP will provide information not captured in earlier steps, especially since the LLNA itself only captures the induction phase of the AOP.

The study of SARs draws heavily on expertise and principles from a wide range of scientific disciplines, and advances in diverse fields of study may significantly impact the utility of (Q)SARs for regulatory toxicology. This is illustrated by the example of QShARs (Box 3.10) as a means of linking atomic knowledge of molecular structure to biological activity.

(Q)SAR is a generally well-accepted technique that is being used in some regulatory risk assessments (reviewed in Cronin *et al*., 2003; Worth, 2010). Indeed, in the EU under REACH, (Q)SARs may be used instead of testing for some chemicals, providing certain conditions are met (European Union, 2006). Limitations to the wider implementation of (Q)SARs centre primarily on the confidence in their current predictivity, which in turn is limited by the relative lack of experimental data

Box 3.10
QShARs Link Atomic-Level Molecular Shape Detail to Biological Outcomes

QShAR is a modern approach to detailed computer modelling and prediction of the biological activity of various chemicals (Mezey, 1993, 1998a, 1998b, 2003; Mezey & Walker, 1997; Mezey *et al*., 2001; Mezey *et al*., 1996; Mezey *et al*., 1998). It is based on replacing the graph-theoretical and similar structure descriptors in conventional (Q)SAR by rigorous molecular shape descriptors (hence "structure" is replaced by "shape" and (Q)SAR is replaced by QShAR). The molecular shape descriptors are based on the computation and analysis of the shape of molecular electron density, which can be accomplished rapidly and accurately with the chemical modelling methods and computer programs developed in the last decades.

The Hohenberg-Kohn Theorem (Nobel Prize 1992, Walter Kohn) asserts that all information a molecule carries is in the electron density cloud of a molecule (Hohenberg & Kohn, 1964). Molecular graph, the basis of conventional (Q)SAR, is merely a molecular skeleton, whereas the electron density cloud is the actual, complete molecular body. Molecular skeleton models, such as graphs, cannot carry the same amount of information as the full electron density cloud, so the latter is far more useful. The (Q)SAR graphs contain very limited information, a few bits represented by the adjacency matrix of the graph, whereas the shape of the electron density in QShAR contains the complete continuum of molecular information; hence, it is far more effective and useful. Furthermore, the Holographic Electron Density Theorem (Mezey, 1999) provides a very practical safeguard for QShAR analysis: it has been proven that any positive volume part of the "body" of the electron density cloud also contains the complete molecular information (Mezey, 1999).

Consequently, even if the mechanism of a particular molecule (such as a pesticide) in any adverse environmental effect is unknown, and if the active region of the molecule is not yet recognized, this is not as seriously limiting as previously assumed. Any part of the molecule — even those not directly involved in the activity — contains all the relevant information and can be used in a shape-activity correlation study.

The principle of QShAR is equally applicable to any molecular family involved in similar biochemical activity, for example, novel drug candidates and herbicides.

on which these predictions are based. Confidence and applicability of (Q)SAR methods could be improved with the ongoing generation of data; standardized and validated models/approaches; open access to data and modelling tools; and a firm understanding (by both researchers and regulators) of the appropriate use and limitations of the tools. The OECD published documentation to address this issue, *Guidance on Grouping of Chemicals* (OECD, 2007f), which uses the categorization principles discussed earlier. The OECD has developed an online (Q)SAR Application Toolbox to facilitate the adoption of (Q)SAR technology and reduce infrastructure costs surrounding its implementation (OECD, 2007b) (Box 3.11).[88]

Box 3.11

CASE STUDY: The OECD Toolbox — Developing (Q)SAR Chemical Categories

The main goal of the OECD (Q)SAR Application Toolbox is to use structure-activity methodologies to group chemicals into categories and provide an *in silico* complement to experimental testing in order to fill existing data gaps.

The key step in using the Toolbox is the formation of a chemical category or "SAR" cluster, which is a group of chemicals whose physicochemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity (reviewed in Bassan & Worth, 2008). The value of a category of chemicals is that members of the category may show qualitatively similar — albeit quantitatively distinct — biological effects presumably based on a common mechanism of toxic action. Data on tested chemicals in the category are used to estimate data for non-tested chemicals in the same category; however, it must be stressed that the accuracy of such predictions varies greatly depending on the type of chemical, type of SAR clustering, and type of biological effect being predicted.

An example of this approach is the study of organic genotoxic carcinogens, which disrupt normal cellular processes and cause abnormal cell growth or tumour development. The OECD Toolbox uses pre-coded structural profiling methods to predict and categorize DNA-binding compounds based on the physicochemical

continued on next page

88 The Toolbox is freeware available from: www.oecd.org/env/existingchemicals/(Q)SAR

Box 3.11 *(continued)*

properties of both DNA and known DNA-binders. Furthermore, it considers not only the parent compound (i.e., the molecule that is in question) but the DNA-binding potential of predicted metabolic products. In this way, the Toolbox endeavours to promote category formation based on DNA-binding. Where possible, the Toolbox uses multiple profiling methods in tandem to provide a transparent, mechanistic basis for categorization with a high level of confidence. The Toolbox could also help populate chemical categories (by finding appropriate analogues) and provide evidence to support category development (e.g., similarity of mechanisms of action or similarity of functional groups) (OECD, 2009b).

3.3.3 Physiologically Based Pharmacokinetic Modelling

As discussed in Chapter 2, the use of *in vivo* animal toxicity data in human health risk assessment necessitates extrapolations across species, exposure routes, exposure durations, and exposure levels. Physiologically based pharmacokinetic (PBPK) models use physiological, biochemical, and physicochemical data to inform these extrapolations in a scientifically robust manner (Thompson *et al.*, 2008). These models can be used to calculate tissue doses for low-dose exposures in different species and in vulnerable subpopulations within a species (Andersen, 2003), making them particularly useful in regulatory risk assessments (Figure 3.13) (Andersen & Dennison, 2001). Indeed, PBPK models have been used by regulatory agencies to predict internal doses at target organs in order to inform risk assessments and improve the scientific basis for subsequent decision-making (for example, see reviews in DeWoskin *et al.*, 2007; Thompson *et al.*, 2008; US EPA, 2006).

PBPK modelling is a computational approach that considers the physiology and anatomy of the body as well as the kinetic character of major biotransformation pathways determined *in vitro*. This combination of physiological and pharmacokinetic information allows the prediction of the concentrations of parent compound and major metabolites *in vivo* (reviewed in NRC, 2006a; Thompson *et al.*, 2008). This approach is often used for species-to-species comparisons in toxicity testing because metabolism varies both qualitatively and quantitatively across species.

The evolution and development of PBPK models has been an interdisciplinary endeavour, influenced by advances in numerous disciplines including biology, chemical engineering, and computer science. The underlying principle for

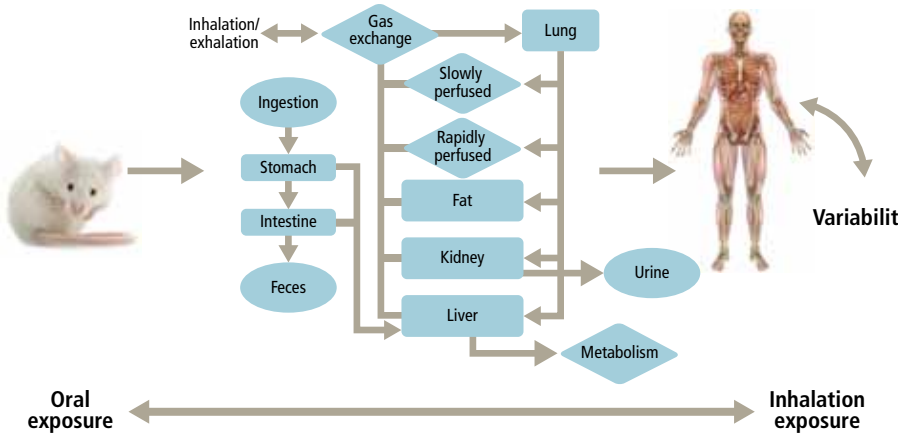


Figure 3.13

PBPK models may derive human reference values from animal toxicity data

modelling the pharmacokinetic properties of a substance, first defined by Sarrus and Rameaux in 1838, relies on the relationship between physiological parameters and body size/weight (reviewed in Heusner, 1991). Subsequent developments have accommodated advances in physiology related to metabolic clearance and dose-dependent metabolism and toxicity, which have contributed significantly to the utility of these models in regulatory risk assessment (reviewed in Andersen, 2003). PBPK modelling can incorporate model parameters and data from a variety of sources — including *in vivo*, *in vitro*, and *in silico* studies (Nestorov, 2007; van de Waterbeemd & Gifford, 2003) — into a comprehensive PBPK model that may help identify links between tissue concentration and pharmacological effects (Espie *et al.*, 2009).

PBPK models that correlate route-of-exposure data with results from biomonitoring studies have considerable promise as tools to facilitate the evolution of biomarkers (reviewed in Lu *et al.*, 2010). In this regard, PBPK models hold considerable promise as a means of linking data derived from population-level studies to those derived from *in vivo* tests. Indeed, the Stochastic Human Exposure and Dose Simulation (SHEDS) model for multimedia, and multiroute chemical exposures is an example of a probabilistic model designed to simulate aggregate and cumulative human exposures (Geller, 2010).

The SHEDS-Multimedia model is designed to incorporate information from a variety of sources — including diary surveys, census data, environmental residues and concentrations, and human activity data — in order to calculate an exposure or dose profile for an individual and an estimate of exposure and dose for a defined population.

The output of the SHEDS model can be used as an input for sophisticated PBPK models in order to model and estimate tissue burden and urinary concentrations in exposed individuals. This was recently shown in a study that simulated residential and dietary exposures to permethrin in 8,994 individuals (Box 3.12).

Box 3.12
CASE STUDY: Use of PBPK to Simulate Population-Level Effects

The metabolic breakdown products of pyrethroid insecticides have been used as biomarkers of exposure in population-level studies, including the 1999–2002 National Health and Nutrition Examination Survey (NHANES). This survey measured the urinary concentration of several pyrethroid metabolites, including *cis*- and *trans*-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (DCCA), which is the urinary metabolite associated with the insecticides permethrin, cypermethrin, and cyfluthrin (CDC, 2009).

The data from the NHANES survey were recently used to test the predictive power of the SHEDS dietary model. The exposures predicted by the SHEDS-PBPK model were compared to NHANES DCCA urinary concentration data.[89] Although the study was preliminary in nature, the correlation between exposure predicted by the SHEDS-PBPK simulation and actual urinary concentrations from the NHANES database was remarkably strong, which highlights the potential for PBPK models to link laboratory simulations to population-level outcomes (Tornero-Velez *et al*., 2010).

Considerable progress has been realized in the development of PBPK models that reflect advances in pharmacokinetics. These models can address animal-human extrapolations, variability for internal dose, and overall uncertainty (Barton *et al*., 2007). Furthermore, the integration of mathematical operators to characterize and

89 The NHANES database of urinary DCCA concentration does not include corresponding data on exposure.

address inter-individual variability and dose-response relationships will increase the reliability and utility of these models (reviewed in Kavlock *et al.*, 2008).

As is the case for any predictive tool, the power of PBPK modelling is enhanced significantly by the availability of scientifically valid data on the physicochemical properties of a chemical. This is an area that might be significantly enhanced by the availability of high-throughput assays that would facilitate the rapid screening of chemicals for a variety of toxicologically relevant properties (van de Waterbeemd & Gifford, 2003). Furthermore, PBPK approaches are relatively data- and resource-intensive. The Panel anticipates that *in silico* strategies would be used at the screening level while higher-tier assessments would use PBPK modelling to increase the accuracy and reduce the uncertainty in the assessment.[90]

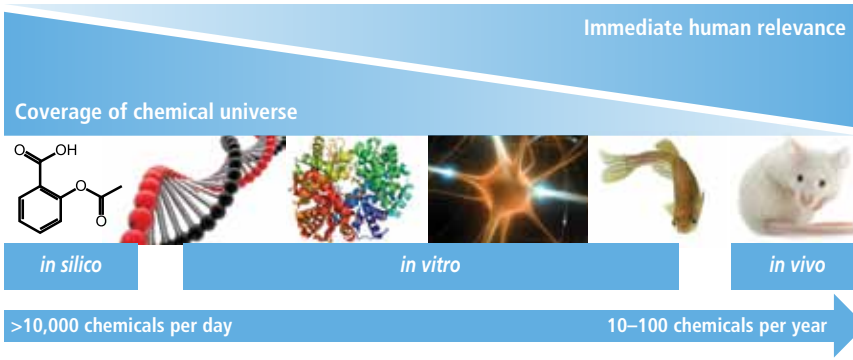
3.3.4 High-Throughput Screening (HTS) for Regulatory Toxicity Testing

Current approaches to regulatory toxicity testing rely principally on the high-dose administration of test chemicals to whole animals (as discussed in Chapter 2), which has the advantage that the chemicals are examined in physiologically relevant contexts.[91] This may be considered "low-throughput screening" since it is capable of examining only a few hundred chemicals per year (and is thus typically restricted to evaluating pesticide active ingredients and pharmaceuticals). In contrast, HTS is a laboratory-based approach that could handle the parallel testing of more than 100,000 chemicals per day, in miniaturized format, for their ability to affect the function of a gene, protein, cell, or model organism. As such, it allows the rapid screening and prioritization of the numerous industrial chemicals for which almost no toxicity testing is currently conducted.[92] HTS relies heavily on robotics systems that have facilitated the automation of entire experimental systems, from sample preparation through to data collection. Between these two extremes are testing systems that are intermediate in complexity and throughput, capable of testing thousands of chemicals per day in complex cellular systems (e.g., electrical activity in primary neuronal cells) or simple model organisms such as the nematode worm *Caenorhabditis elegans*. In considering these approaches, the trade-off between experimental throughput and human relevance must be kept in mind (Figure 3.14).

90 The Scientific Advisory Panel of the US EPA is currently reviewing the use of PBPK modelling for higher-tiered risk assessment: http://www.epa.gov/scipoly/sap/meetings/2010/072010meeting.html

91 The Panel acknowledges that the physiology of the laboratory test animal may be quite different from that of a human, leading to inaccurate extrapolation and interpretation of observations.

92 The Panel acknowledges that the effects induced by a chemical in an *in vitro* assay may not reflect those induced in a living organism.



(Adapted and reproduced with permission from *Science* and Collins *et al.* 2008)

Figure 3.14
The inverse relationship between immediate human relevance and experimental throughput

HTS originated in the pharmaceutical industry (Pereira & Williams, 2007) where it is widely used to systematically test millions of small molecules to identify candidates for lead optimization using medicinal chemistry. As of 2005, the US Tox21 Consortium (Box 3.13) adopted HTS approaches to identify toxicologically significant cellular responses after exposure to chemical compounds (Collins *et al.*, 2008; Kavlock *et al.*, 2009).

While HTS is well established in drug discovery and is a promising component of an integrated testing strategy, its use in toxicology is in its infancy. Its strengths and limitations may be unfamiliar to toxicologists and risk assessors, and so will be reviewed here. There are important differences in the use of HTS for toxicity testing relative to drug development (Box 3.14). In drug development, HTS tests compounds at a single concentration (generally 10 μM); however, it is a basic tenet of pharmacology and toxicology that the observed biological effect of a chemical is dependent on concentration (or, when exposure to a living organism is considered, its "dose"). Thus, single-concentration HTS produces high rates of false-positives (up to 95 per cent) and false-negatives (up to 70 per cent). Although undesirable, this may be more readily tolerated in drug discovery; however, it is not acceptable when used for toxicological evaluation or prioritization where chemicals must be more accurately profiled. Partly to address the needs of toxicological profiling in the pilot phase of the Tox21 program, the NIH Chemical Genomics

Box 3.13
CASE STUDY: The Tox21 Consortium

The challenge of integrating scientific advances into regulatory toxicology is not trivial. Individually, the National Toxicology Program (NTP, 2004), the NIH Chemical Genomics Center (Austin *et al*., 2004), and the US EPA (US EPA, 2009q) recognized the need to bring innovation into the assessment of chemical hazards and risk.

In February 2008, following the release of the 2007 NRC report *Toxicity Testing in the 21st Century: A Vision and A Strategy*, these three U.S. governmental agencies entered into a Memorandum of Understanding (Tox21, 2008). Their joint aim is to bring their expertise and complementary capabilities to bear on transforming the conduct of toxicological evaluations (Collins *et al*., 2008). In June 2010, this MOU was expanded to include the US Food and Drug Administration (FDA) (Tox21, 2010).

Four working groups operate within Tox21: chemical selection, assay selection, informatics, and targeted testing (Kavlock *et al*., 2009). Initially the NTP and US EPA each contributed approximately 1,400 chemicals to an assay program focused primarily on nuclear receptor and other cell signalling biology. This effort proved that quality data could be obtained through HTS approaches, and examples of these are starting to appear in the literature (Xia *et al*., 2009). Currently the consortium is developing a library of over 10,000 environmental and pharmaceutical chemicals, with screening scheduled to start in mid-2011 (Kavlock, personal communication).

Center developed concentration-response-based HTS, termed Quantitative High-Throughput Screening (qHTS) in 2006 (Inglese *et al*., 2006). The application of qHTS to toxicology-relevant endpoints is now well established (Huang *et al*., 2008; Shukla *et al*., 2010; Xia *et al*., 2008).

The three primary strengths of HTS are throughput, utility to deduce compound mechanisms, and use of human (rather than model animal) materials in testing. The throughput of HTS depends principally on the incubation time of the assay and the complexity of the readout. For simple readouts, such as enzyme activity or acute cytotoxicity, HTS is capable of testing over 100,000 chemicals at seven or more concentrations in a single day. To put this into perspective, this is equivalent to testing all chemicals in commerce — or more chemicals than

Box 3.14
CASE STUDY: Key Differences Between Toxicity Testing and Drug Development

Many of the HTS assays and tools were initially developed to support drug development; however, there a number of important differences between drugs and environmental chemicals.

Toxicity screening for pharmaceuticals versus environmental chemicals

	Pharmaceuticals	Environmental Chemicals
Chemical space	Narrow	Broad
Number of chemicals	$10^4 – 10^6$	$10^2 – 10^4$
Intended MoA	Generally known and specific	May not exist
Target potency	High	Generally low
Error tolerance	False positives can be problematic	False negatives not acceptable

(Dix *et al.*, 2007)

Drugs are developed with discrete biological targets in mind; have relatively high target molecule affinities; conform to a limited range of physical-chemical properties (e.g., Lipinski's rules); have well-understood metabolic profiles; and have known and quantified patterns of use.[93] Many of the tools used in computational toxicology were developed with these aspects in mind, but had to be adjusted to the broader structural universe of environmental chemicals. With the exception of pesticides, environmental chemicals may not have discrete intended biological targets; do not exhibit high affinity interactions with molecular targets; have largely unknown metabolic patterns; and have highly variable patterns of use that may result in highly variable exposures.

have been tested in the entire history of toxicology testing.[94] Furthermore, this testing can be done on human cells or proteins — making organismal relevance more immediate — and biological mechanism information is inferred directly from the HTS, since the assays can be designed to indicate as narrow or broad a mechanistic examination as desired.

93 Lipinski's rules are used to evaluate the likelihood that a chemical compound that exhibits certain pharmacological or biological activities is likely to be orally active in humans (Lipinski *et al.*, 2001).

94 Although this is against a single target and there are many targets. Further research is needed to identify those targets that are currently unknown.

This tremendous throughput and mechanistic information is, however, balanced by equally important drawbacks. The three primary weaknesses of HTS are lack of tissue or organismal context; absence of exposure (route, extent) or (for the most part) metabolic capacity; and the limitation of effects to a single, easily cultured cell type.[95] HTS for toxicology testing can be conceptualized as testing chemicals on the individual pathways and cells that make up the organism rather than the intact organism itself. The conclusions about whole organism effects are inferred from the computational integration of many cell-level results (a "bioactivity signature") and comparison to whole-animal results on structurally related chemicals.

This approach has intuitive appeal, and recent studies have highlighted its promise as a means of identifying bioactivity signatures that relate exposure to a chemical with a toxicological outcome (R. S. Judson, Houck *et al.*, 2010; R. S. Judson *et al.*, 2011; Martin *et al.*, 2010). Nevertheless, the ability of reductionist (structure-, cell-, or pathway-level) testing to predict whole-animal or human toxicity remains unproven. The development of HTS-driven and computational toxicology is at an early stage, and *in vitro*-derived bioactivity signatures and computational models are being tested for their predictive capacity (Box 3.15). While there is good reason to be optimistic, the history of cell-based screening and (Q)SAR in the pharmaceutical industry indicates that results in cells will not always translate to results in humans, any more than effects in laboratory animals translate to effects in humans. In both cases, healthy scepticism and rigorous testing of assumptions and hypotheses will be key to proper implementation for risk assessment.

Box 3.15
CASE STUDY: ToxCast™

ToxCast™ is a multi-year, multi-million dollar effort to apply batteries of *in vitro* tests on chemicals that have already been evaluated using traditional *in vivo* studies for cancer, reproductive impairment, and developmental disorders. Committed to transparency and the public release of all data, it is the most strategic and coordinated public sector effort to transform toxicology.

The goal of ToxCast™ is to generate sufficient data on a broad range of chemicals to permit the identification of "bioactivity signatures" that correlate cellular responses to the organismal outcomes observed in traditional toxicity testing (Kavlock *et al.*, 2007). Bioactivity signatures include responses at levels of biological organization

continued on next page

95 Considerable research is currently underway to provide solutions that can address these limitations.

Box 3.15 *(continued)*

below that of the outcome of regulatory interest. This allows tissue and organ responses to be used as bioactivity signatures for whole-organism responses. These predictive bioactivity signatures are based on a broad suite of information including physicochemical properties; SAR models; genomic analyses of cells *in vivo*; apical outcomes observed in non-mammalian model organisms; and *in vitro* data from HTS and cell-based phenotypic assays.

Phase 1 of ToxCast™ involved the evaluation of 309 unique chemicals against a battery of 467 *in vitro* assays from different technology platforms. Results of the first phase of ToxCast™ (R. S. Judson *et al*., 2009) demonstrated a broad spectrum of chemical activity at the molecular and pathway levels, with chemicals interacting with a mean of about 50 assays and some with more than 100 assays. Many of the expected interactions were seen in the data, including endocrine effects and xenobiotic metabolism activity. When assays were mapped to biological pathways, chemicals showed widely varying promiscuity across pathways, from no activity to activity against dozens of pathways. Interestingly, there was a statistically significant inverse association between the number of pathways perturbed by a chemical at low *in vitro* concentrations and the lowest *in vivo* dose at which a chemical first causes toxicity.

The ToxCast™ chemicals were largely derived from a list of food use pesticides, and hence are generally regarded as non-genotoxic chemicals; however, 21 of the 309 chemicals were shown to induce liver tumours in rats after chronic exposure. This bioactivity signature suggests that if a chemical that interacts with the peroxisome proliferating-activated receptor gamma pathway (PPARγ) and one or more other key pathways, there is a significantly increased likelihood for inducing liver tumours in rats when compared to non-genotoxic chemicals activating none or only one of these processes (R. S. Judson *et al*., 2009).[96] The National Toxicology Program of the National Institute of the Environmental Health Sciences (NIEHS) is currently testing this prediction model.

ToxCast™ is now entering Phase 2, which will examine the effects of an additional 700 chemicals against a similar range of assays. These will include data-rich food-use pesticides, a number of drugs that failed during human clinical trials, representatives of several categories of HPV chemicals, and data-rich food additives. The diversity of these chemicals reflects the need to adequately characterize the full spectrum of environmental chemicals that ToxCast™ assays must ultimately be able to screen and prioritize.

96 These key pathways are PPARγ activation, cytokine CCL2 up-regulation, androgen antagonism, or oxidative stress.

Simmons *et al*. (2009) reviewed a number of particular approaches to examining cellular stress responses for use in toxicological screening. They highlighted that, while pharmaceutical agents exert their effects via specific targeted mediated alterations, environmental toxicants are likely to cause toxicity by more generalized mechanisms and pathways. They hypothesized that evaluating cellular stress pathways would provide sentinel observations of key modes of action. There are a limited number of cellular response pathways that could be activated in a cellular autonomous fashion and also participate in cell fate decisions such as apoptosis. These pathways are common to nearly all types of cells in nearly all mammals. They are fundamental to cellular fate and survival and include those pathways involved with responding to oxidative stress, heat shock, DNA damage, hypoxia, endoplasmic reticulum stress, metal stress, inflammation, and osmotic stress. Experiments with transgenic and knockout animals indicated the importance of these pathways in developmental processes and in disease progression. An approach similar to the one described by Simmons *et al*. (2009) was used against a small number of chemicals using a yeast-based reporter system (Dardenne *et al*., 2008); it successfully grouped chemicals by known MoAs, demonstrating the feasibility of broad spectrum biological profiling using *in vitro* assay systems.

The adoption of HTS in regulatory toxicology might proceed in a two-pronged fashion that maximizes both the number of chemicals assayed and the breadth of assays used. The analysis of a small group of chemicals against a large number of targets would permit the identification of key toxicity pathways. The analysis of a large number of chemicals against a small number of targets would permit the proof-of-concept demonstration of the utility of HTS to specific applications. This kind of synergistic approach should result in the development of an informative and biologically-based process for the screening and prioritization of chemicals.

Human Tissue Culture Cell Lines, Stem Cells, and *in vitro* Testing:

Assays for screening of toxicologically relevant responses should be done *in vitro* with human cells that are as representative of an *in vivo* human tissue as possible. To this end, cell-based assays for use in toxicity testing have been under development for a number of years; however, cell lines used in classical tissue culture exhibit significant limitations in this regard, compromising their relevance to toxicity testing.[97]

97 Transformed human cell lines used in classical tissue culture are usually derived from cancer cells and possess several abnormal characteristics such as aneuploidy, reduced functional properties, and a limited phenotype of the cells they originated from. Such cell lines may not respond to pesticides and other chemicals in a normal fashion. Similarly, immortalized or neoplastic cell cultures contain cells that exhibit "stem-like" properties (i.e., unlimited proliferation potential); however, any cell line is purely clonal so issues of genomic and epigenetic instability that are pervasive in neoplastic cells pose considerable problems in assay development.

Furthermore, the two-dimensional (2-D) culture systems in which these cells are grown do not mimic the conditions of the *in vivo* microenvironments. 2-D cell culture techniques typically use monolayer cultures grown in petri plates. 3-D cell culture techniques use methods that permit the aggregate growth of cells, which more closely mimics physiological conditions.

HTS requires cells that are robust, have unlimited capacity for self-renewal, and closely imitate the behaviour of normal cells *in vivo* when assayed. Primary cells from human donors may be suitable in some settings, but with few exceptions they have limited ability to self-renew and may lose cell-type defining characteristics within 24 hours *in vitro*. Stem cell-derived cells have recently been identified as promising candidates for future toxicity testing in *in vitro* systems (Chapin & Stedman, 2009) because they are capable of self-renewal and exhibit pluripotency (Nirmalanandhan & Sittampalam, 2009).[98] Nonetheless, considerable work is still needed to characterize the cells derived from stem cells and show their similarity to native cells.

Although the development of stem cell-based high-throughput assays for toxicity testing is in its infancy, there is considerable work underway in this area. The murine embryonic stem cell test (EST) (Box 3.16) is one example of an *in vitro* screening tool that could be used to classify chemicals based on their development toxicity potential. This test has seen some use in the pharmaceutical industry (Paquette *et al.*, 2008). The EST was developed at the Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET) at the Federal Institute for Risk Assessment (BfR) in Berlin, Germany, and was validated by the European Centre for the Validation of Alternative Methods (ECVAM), which is part of the European Commission Joint Research Centre, in Ispra, Italy.[99] Since then, it has been the subject of considerable scrutiny, and is a good illustration of the challenges inherent in validating such approaches (Daston *et al.*, 2010). For example, the endorsement of a test as scientifically valid is distinct from regulatory acceptance of that test as a suitable replacement for an existing assay (the issue of validation will be discussed in more detail in Chapter 4.). The EST was not validated for use as a replacement for developmental toxicity tests, and the ECVAM website states that OECD adoption is not foreseen.

98 These cells can give rise to all three embryonic layer types: ectoderm, endoderm, and mesoderm from which all tissues and organs subsequently derive in the developing embryo.

99 The EST was also part of the ReProTect battery, which recently published a successful prospective study (Schenk *et al.*, 2010).

Box 3.16
CASE STUDY: The Embryonic Stem Cell Test

The Embryonic Stem Cell Test (EST) is used to screen chemicals for their potential inhibitory effect on the *in vivo* differentiation of embryonic stem cells into myocardial cells. Myocardial cells were selected because cardiomyocytes represent one of the first functional organ-like systems in the developing embryo. Furthermore, they are easy to identify because they exhibit contractile activity (Spielmann *et al*., 1997). The EST used a set of 20 reference compounds with known teratogenic properties. An overall score of 78 per cent was obtained using this reference set, with a correct classification of 100 per cent for those chemicals known to exhibit strong embryotoxic properties (Genschow *et al*., 2004). By comparison, the micromass test and the post-implantation rat whole embryo culture assay (i.e., the traditional *ex vivo* tests) gave 70 per cent and 80 per cent correct classifications, respectively (Genschow *et al*., 2004; Piersma, 2004; Spielmann *et al*., 2004).

In 2001, after prevalidation and validation studies (Genschow *et al*., 1999; Scholz, Genschow *et al*., 1999; Scholz, Pohl *et al*., 1999), ECVAM endorsed the EST as a valid *in vitro* method for the detection of embryotoxic hazards (Genschow *et al*., 2002). At the request of the validation committee, a follow-up workshop was held, which led to the recommendation that further tests be developed before the EST could be used for regulatory purposes (Spielmann *et al*., 2006).

The EST correctly classified lithium and hexavalent chromium as embryotoxic metals and trivalent chromium as non-embryotoxic (Genschow *et al*., 2004; Stummann *et al*., 2007). Conversely, the EST has misclassified several compounds of embryotoxicants (cadmium, arsenite, and arsenate) (Stummann *et al*., 2008), and predicted the strong embryotoxicant methylmercury as non-embryotoxic in four out of eight experiments (Genschow *et al*., 2004). The EST represents a relevant *in vitro* screen for the prediction of embryotoxic potential of direct-acting teratogens; this can lead to misclassification when testing pro-teratogens, due to their need for metabolic activation (Hettwer *et al*., 2010; Marx-Stoelting *et al*., 2009).

An embryotoxicity test incorporating a metabolic activation system consisting of isolated primary hepatocytes is now available. A co-culture system has been developed where the test compound is incubated with hepatocytes, and the supernatant of the hepatocytes culture is added to the embryonic stem cell culture (Hettwer *et al*., 2010). Due to interspecies differences in the bioactivation of a number of pro-teratogens, the EST is optimized to take into account human metabolism by the addition of metabolizing enzymes.

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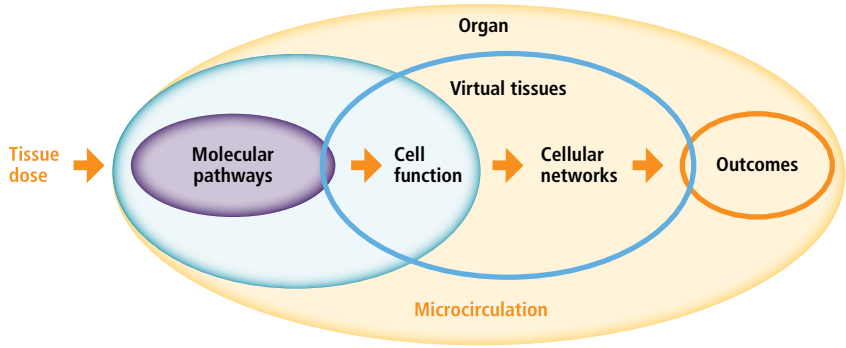
Box 3.16 *(continued)*

Adaptation of the EST for developmental neurotoxicity testing is an emerging research domain, and the issues associated with health development versus neurodevelopmental biology (as well as cardioteratogenicity and neuroteratogenicity) must be addressed. The identification of neural stem cell markers and differentiation markers for different neuronal stages is required. Refinement of the EST prediction model and inclusion of additional toxicological endpoints could expand the predictive power of the test for metals. Whether the adaptation of the EST for metals will require the identification of specific endpoints remains to be seen.

3.3.5 Building Virtual Tissues

As discussed earlier, PBPK models may be used to explain a relationship between external exposure and internal tissue dose. The utility and predictivity of PBPK models are likely to evolve as the state of scientific understanding continues to increase. In the long term, the development of multi-scale models (or "virtual tissues") may augment PBPK models by bridging findings from high-throughput *in vitro* screens to the pathologic sequences occurring *in vivo* across chemical, dose, time, and species (Figure 3.15) (Shah & Wambaugh, 2010).



(Reproduced with permission from Taylor & Francis Group)*

Figure 3.15
The modular architecture of virtual tissues

*Virtual Tissues in Toxicology, Imran Shah, John Wambaugh, *Journal of Toxicology and Environmental Health*, Part B: Critical Reviews, Jan. 2, 2010 Taylor & Francis. Reproduced with permission of the publisher Taylor & Francis Group, http://www.informaworld.com

The goal of these models is to predict histopathological outcomes from alterations of cellular phenotypes that are controlled by underlying regulatory networks (Box 3.17). This is similar to the directions being explored in medical diagnostics and therapeutics.

Box 3.17
CASE STUDY: Developing a Virtual Liver

The liver is a frequent target of chemicals, and much is known about hepatic metabolism, gene regulatory networks, and progression of altered function and disease. It represents a logical starting point for the development of toxicologically relevant virtual tissues. Wambaugh and Shah (2010) began developing a virtual liver by computationally representing the delivery of blood to the hepatic lobule and providing a description of the dose or exposure of the liver to the toxicant.

The mammalian liver is composed of approximately one million functional units (lobules) that receive blood from up to six portal triads (each composed of a hepatic arteriole, a portal venule, and a bile ductile). Blood flows from the triad into the sinusoidal space between hepatocytes and drains into the central vein. The hepatocytes are arranged in plates of one-to-two cell thickness around the central vein. As blood moves through the sinusoids, the hepatocytes can uptake, metabolize, and secrete chemicals (including nutrients and xenobiotics) carried by the blood.

To build the model, Wambaugh and Shah (2010) first approximated the microanatomic architecture of the hepatic vasculature and parenchyma using a connectivity graph that assumed a discrete topology. A simplified geometry of the lobule was defined using the number of portal triads, the branching factor of the sinusoids, the number of sinusoids entering a central vein, and the sizes of sinusoids, hepatocytes, and lobule. The graphical model was then iteratively constructed using those parameters and visualized spatially. Small random variations in the placement of branching of the sinusoidal primitives were sequentially used to reconstruct the histological appearance of the lobule. Hepatic arterioles and portal venules were placed at the perimeter of the lobule, and parenchyma cells were placed contiguously with the sinusoidal network.

A simple agent-based model was used to describe hepatocyte responses. In this implementation, each hepatocyte was defined by fixed, identical xenobiotic metabolism rates as well as functional states that were updated each time-step according to

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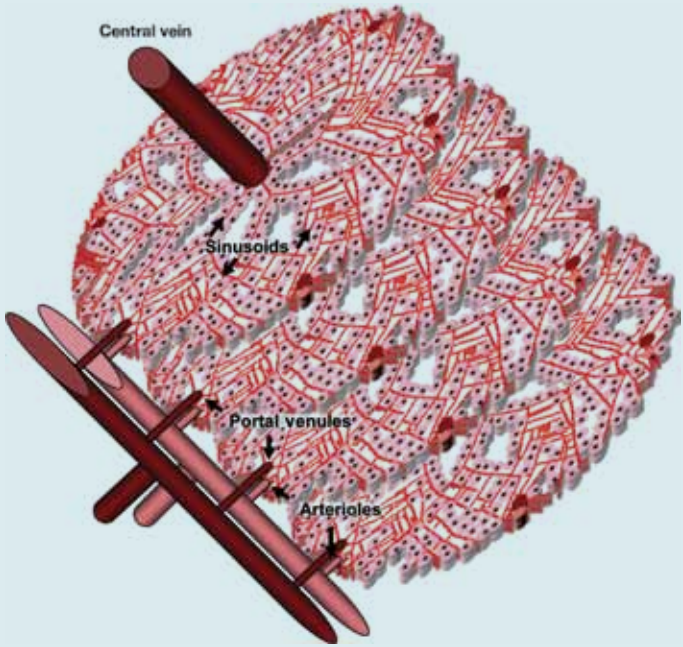
Box 3.17 *(continued)*



(Reproduced with permission from Wambaugh & Shah, 2010)

Visualization of the first generation virtual liver showing the portal triads, hepatocyte-lined sinusoids, and the central vein

state transition rules. Next, they transformed the sinusoidal elements of the vascular network into a system of well-mixed compartments through which one-dimensional flow occurred. Mass transfer through the sinusoidal network occurred along the edges. Finally, they connected the virtual lobule to a PBPK model to provide systemic exposure. When tested with a rapidly metabolized chemical, an increase in parent compound concentration heterogeneity across the vascular network translated to a greater variability in cellular response of apoptosis.

With the availability of physiologically representative models of hepatic microdosimetry, the virtual liver is now poised to incorporate more biologically realistic aspects of hepatocellular molecular dynamics (for example, the role of nuclear receptor activation on induction of hepatocyte proliferation and liver tumour induction), including the incorporation of interacting cell types such as the Kupffer cells.

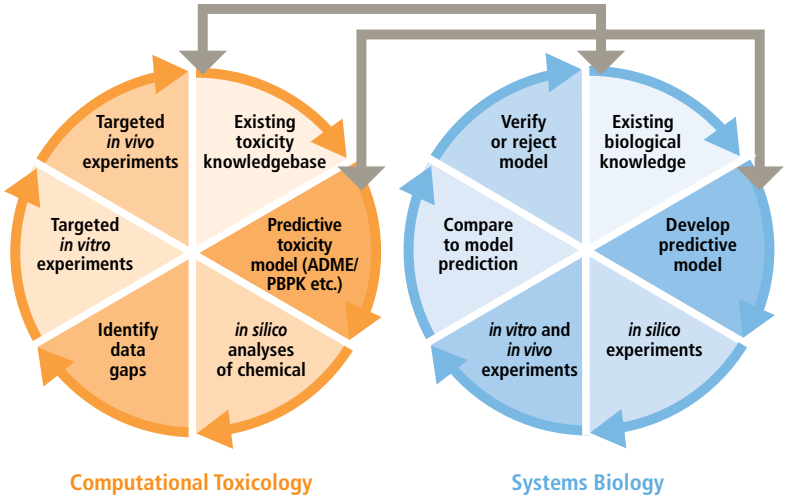
Development of virtual tissue models for toxicology represents a long-term aspiration; doing so will require advances in computational analyses of histological images to extract microanatomical features and to describe cellular heterogeneity. Knowledge-based mechanistic modelling can help provide descriptions of experimental evidence and theories in computer-readable form. A challenge here is the need to standardize biological concepts and to curate them from the scientific literature. The advantage of the models will be the translation of *in vitro* high-throughput assay results into tissue-level responses. Once the existing knowledge is captured, it will be translated into a multi-scale model using approaches such as agent-based models that integrate tissue microdosimetry, molecular regulatory networks, and cellular behaviours. Although there are major challenges in implementing such models, once developed, they could help to characterize exposures in target tissue and explore various exposure patterns in terms of response at the cellular level. They could also predict human response from pathway perturbations and quantify the range of human variability and susceptibility. As in the biomedical field, considerable efforts will be needed to develop models that are both biologically realistic and sufficiently detailed to be useful for hazard and risk characterizations.

3.3.6 Summary of the Key Toxicity-Modelling Tools

One of the key challenges in developing any predictive toxicology tool is how to integrate the data in order to build a sufficiently comprehensive picture of the physiological response to exposure. HTS and (Q)SAR models may provide starting data for large numbers of chemicals. Existing knowledge of chemicals and toxicological responses, when available, is used to develop predictive models of toxicity that, in turn, may facilitate the *in silico* analyses of new chemicals. When data gaps exist, targeted *in vitro* and *in vivo* experiments may be used (in that order) to generate the necessary data to inform a risk assessment decision. These data would then be entered into the knowledgebase.

Predictive models, as well as the design of appropriate *in vitro* and *in vivo* testing strategies, will benefit tremendously from the input of data from systems biology. For this reason, the Panel believes that systems biology and computational toxicology require parallel development, with knowledge generated in one area being used to inform decisions in the other (Figure 3.16).

The next section of this chapter will review some of the scientific challenges for systems biology and computational toxicology that will need to be addressed by future research projects so as to advance the evolution of systems-level models.



(Adapted and reproduced with permission from Macmillan Publishers Ltd.: Kitano, H. (2002a). Computational systems biology. *Nature*, 420(6912), 206-210, Copyright 2002)

Figure 3.16
Computational toxicology and systems biology use integrated and analogous processes

3.4 SCIENTIFIC CHALLENGES AND RESEARCH OPPORTUNITIES

The scientific challenges inherent in developing an adequate understanding of biological processes are substantial. The Panel has elected to focus on those challenges most relevant to advancing the understanding necessary to build better, more predictive models of toxicity that could be used to evolve and improve the current testing system over the next decade.

Some of these challenges, when overcome, may help to address limitations in the existing toxicity testing system, as described in Chapter 2. Conversely, some of those limitations may not be addressable within the short term. Many of the advances that the Panel believes might take place by integrating IATA into the toxicity testing process, however, may have implications for addressing those limitations over the long term.

3.4.1 *In vitro* and HTS Assay Development

Traditional toxicology using animal-based tests has necessitated using interspecies extrapolation. The use of *in vitro* tests necessitates a different type of extrapolation; how to relate modifications at the cellular level to biologically significant perturbations at the organismal level — *in vitro* to *in vivo* extrapolation. The Tox21 Consortium is addressing these issues (Box 3.13) (Kavlock *et al.*, 2009), but

considerable development work, both to catalogue those pathways and develop assays that target them, will be needed over the coming years. In addition, *in vitro* assays are generally very sensitive; however, activation of *in vitro* endpoints does not necessarily represent an adverse biological response (Rotroff *et al.*, 2010). The development of predictive signatures that would facilitate the use of *in vitro* data to predict *in vivo* responses is underway, but the difference between adaptive and adverse response is contentious (R. S. Judson, Houck *et al.*, 2010).

Identification of Key Indicator Pathways:

It will likely take many years to elucidate and map all the toxicity pathways that provide mechanistic information contextualizing the observable toxicological endpoints. However, periodic reviews of the data generated from HTS assays for all chemicals could help facilitate the identification of emerging patterns of biological activity. These emerging patterns would, in turn, facilitate the identification of a set of indicator pathways whose activation could be correlated with specific toxicological outcomes, which will be important in demonstrating the utility and practicality of *in vitro* tests (Andersen & Krewski, 2009).

Indicator pathways remove the need for a complete and integrated understanding of human and/or ecological systems by identifying specific pathways known to be involved in toxicological responses. These pathways are then examined using a variety of *in vivo* techniques to obtain information about their contribution to toxicological responses and the mechanism by which the response manifests. The US EPA has used this technique for investigating exposure pathways for over 20 years (Jeffrey, 2000).

The question of identifying appropriate indicator pathways is not entirely straightforward; a number of considerations need to be addressed in order to design research programs that can facilitate their elucidation. These include establishing a baseline that defines physiological "normal" and discriminating between an adaptive and an adverse response.

Discriminating between an adaptive and an adverse response at the cellular level is intimately connected to the definition of "optimal health status." Such definitions differ between scientific disciplines because endpoints are not the same; however, it is important to discriminate between toxicity pathways and risk at the cellular level. The assessment of cellular risk does not address probabilistic considerations (Box 3.18). *In vitro* assay systems are not designed to analyze "risk to the cell;" rather, the goal of these assays is to identify molecular "targets." As *in vitro* toxicology tools become more sophisticated, it is reasonable to expect to identify many of the molecular targets that are involved in more than one biochemical

pathway. To work towards elucidating all toxicity pathways will therefore require a detailed and comprehensive understanding integrated across different levels of biological complexity, which is ultimately the goal of systems biology.

Box 3.18
An Aside on Addressing the Concept of Risk at the Cellular Level

At the cellular level, risk refers to the concept of balance (or imbalance) between levels of "action" (or effect) and capacity of mechanisms of protection. This concept of risk should not be confused with regulatory risk assessment, which addresses risk at the level of individuals (in human health risk assessment) and populations (in environmental risk assessment). Similarly, the term "injury" is used only when discussing systems whose protective mechanisms are overloaded. Perhaps the best examples of this can be seen by studying oxidative stress, which arises when pro-oxidative conditions overload physiological oxidative defence mechanisms, or heavy metal toxicity, when, for example, metallothioneins are saturated and additional intracellular metal cannot be inactivated.

Even at the cellular level, perturbation of toxicity pathways does not necessarily result in a toxic effect. The threshold dose at which cellular protection mechanisms are overloaded may differ from the threshold dose for a toxic effect, which directly raises the question of risk estimation and the calibration of *in vitro* responses for *in vivo* relevance.

The Development of Assays based on Mode of Action:

Techniques such as HTS identify biological effects in *in vitro* assays and seek to determine their toxicological relevance (if any) at the organismal or population level. This approach can provide large amounts of information on many substances in a comparatively short time. Over time, it may be possible to identify bioactivity signatures that could help elucidate as yet unidentified MoAs (or AOPs). In turn, MoA (or AOP) studies might use the data and resulting models generated by HTS to establish causal linkages, which would help in the development of new assays that focus on the effects of exposure on specific pathways.

This approach is commonly used to evaluate the MoAs of drugs where bioassays are used to measure the ability of a compound to disrupt intra- or intercellular processes. Research into suitable *in vitro* assays for ascertaining MoA has already begun (reviewed in Edwards & Preston, 2008; Elespuru *et al.*, 2009; Huang *et al.*, 2008; R. S. Judson, Houck *et al.*, 2010; Tong *et al.*, 2009). Nonetheless,

the Panel anticipates that, as toxicity pathways are elucidated, many new assays may be developed in order to fully realize the technological advances arising from genomics, proteomics, metabolomics, and bioinformatics research that have been discussed in this report.

Modelling Dose-Response Relationships:

In order to use *in vitro* data in human health risk assessment, it is necessary to understand both the appropriateness of the exposure level (dose) used and the relevance of the chemical form.

A prerequisite to establishing the appropriate exposure level is knowledge of bioavailability. This varies with exposure route, exposure source, and distribution. Knowledge of biotransformation events that modify chemicals during their distribution to target organs is required in order to understand the relevance of the chemical form.

Although *in vitro* approaches may well contribute to knowledge of bioavailability and distribution, there is a gap between studies designed to estimate toxicokinetic parameters and those designed to evaluate mechanism(s) of toxicity. For *in vitro* cell (tissue) models to be reliable, exposure conditions should mimic, as much as possible, those of the *in vivo* situation. Thus, dose-response relationships derived from *in vivo* data should consider physiological and kinetic data generated from a number of toxicity testing tools. In turn, data obtained from *in vitro* tests that consider these same physiological factors might be more appropriate and useful for PBPK modelling.

Until now, most *in vitro* models have used relatively high concentrations and short exposures; studies that investigate chronic exposure to low doses have not been as numerous. Studies that assess both acute and chronic exposures should be designed such that the expected exposure scenario considers the source, frequency and duration of exposure and the half-life of the chemical agent. The Panel notes that this does pose some challenges in the interpretation of chronic outcomes for *in vitro* assays.

3.4.2 Molecular Epidemiology and the Identification of Appropriate Biomarkers

Genetic epidemiology focuses on the study of genetic variation as a determinant of health outcomes in populations. It also includes the study of the environmental determinants that interact to contribute to these outcomes. It uses tools from population and molecular genetics, epidemiology, and biostatistics. In general, it targets the identification of genetic susceptibility factors in the form of rare or common genetic variants.

In the context of an IATA approach, the results of molecular and genetic epidemiological studies could be very useful during the modelling phase of the risk assessment procedure. Although the population would not yet have been exposed to the agent being studied, it may belong to a family of similar or related components with similar MoAs or AOPs for which population data do exist. The IATA process could borrow from this information to make predictions of the toxicological properties of the studied agent in the risk assessment scenarios. This principle is illustrated in Figure 3.17. Using genetic epidemiological data from structurally related chemicals during decision-making for a new agent under study is a novel suggestion that could enhance the validity of risk assessment predictions for the agent.

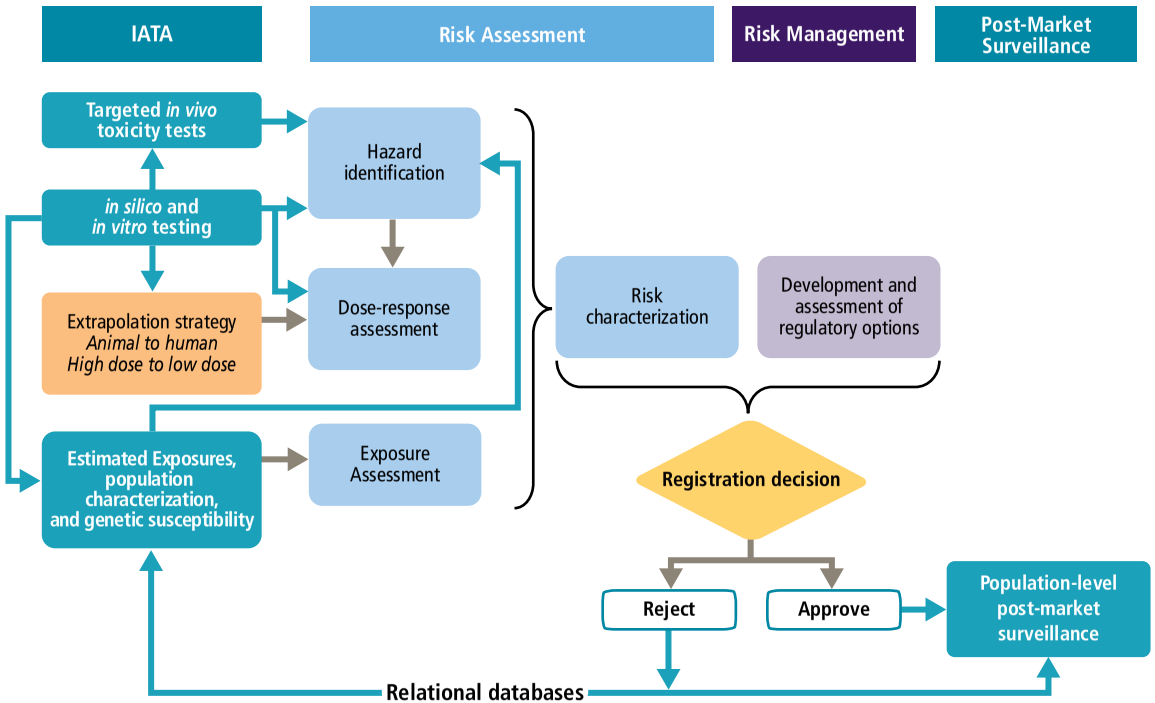
Molecular epidemiology integrates molecular biology with traditional epidemiology in order to develop and identify biomarkers of exposure, susceptibility, effect, or cross-species comparability (Table 3.3).[100] These biomarkers could be used to study genetic risks associated with environmental exposures. The results of these studies would then be used to inform the pre-market risk assessment of structurally related chemical entities (Figure 3.17). In this regard, the development of appropriate biomarkers could greatly enhance both pre-market toxicity testing and post-market surveillance efforts by providing a quantitative estimate of exposure while reducing sampling bias. With respect to post-marketing surveillance studies — since they are both expensive and impractical to measure on a large scale, especially before disease onset — their selection should be based on strong evidence of exposure or effect (Box 3.19).

Table 3.3

Categories of biomarkers of relevant to the regulatory toxicity testing of pesticides

Type of Biomarker	Description	Value
Exposure	Indicate presence of the chemical in an individual, suggesting exposure has resulted in biological interaction	Provide a quantitative means of estimating exposure to a pesticide
Susceptibility	Indicate inter-individual differences that may affect response to environmental agents	Permit refined assessment of risk through identification of gene-gene and gene-environment interactions
Effect	Indicate presence of disease, early disease progression, or events peripheral to a disease process	–
Cross-species comparability	Biomarker to link response in exposed test animal to human response	Reduce uncertainty in inter-species extrapolation

100 The U.S. National Institute of Environmental Health Sciences (NIEHS) defines a biomarker as a *"key molecular or cellular events that link a specific environmental exposure to a health outcome"* (NIEHS, 2010).



(Adapted and reproduced from Risk Assessment in the Federal Government: Managing the Process, 1983 with permission from the National Academy of Sciences, Courtesy of the National Academies Press, Washington, D.C.)

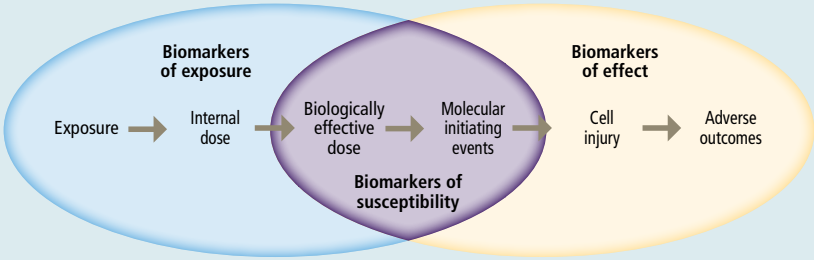
Figure 3.17

Incorporation of epidemiology into the pre-market risk assessment process

This process is an expanded version of the figure that appears in Box 2.2

Box 3.19
CASE STUDY: Biomarkers of Exposure to, and Effect of, Organophosphates

In order to study the relationship between exposure and response, biomarkers must be measurable indicators that can be directly linked to the event in question and can be representative of exposure, effect, or susceptibility (Timbrell, 1998). An ideal biomarker would be readily measurable and quantifiable as well as possess a high degree of sensitivity and specificity. For toxicological studies, biomarkers are used as a method for identifying and studying the effect of exposure to given entities on specific signalling pathways (Tugwood *et al*., 2003). In this way, they serve as "trackers" of biological responses and can be used to track each phase of the dose-response continuum (Schmidt, 2006), assuming that that pathway has been characterized.



(Council of Canadian Academies)

Biomarkers can be used to track events along the continuum from exposure to outcome

For example, knowledge of the pathways by which organophosphates may be degraded in the human body has permitted the development of specific biomarkers of exposure to several organophosphate pesticides (Leng & Lewalter, 1999). Although these biomarkers have predominantly been used to assess exposure in agricultural workers (for example, Hofmann *et al*., 2010; Hofmann *et al*., 2009), they have also been used in a wide variety of other sub-populations — including children (Curwin *et al*., 2007) and pesticide-manufacturing workers (Leng & Lewalter, 1999) as markers of recent exposure. Biomarkers of biologically persistent organochlorines have also

continued on next page

Box 3.19 *(continued)*

been developed and used in studies of cancer and other chronic health outcomes (for example, Spinelli *et al*., 2007). Although reconstruction of exposure on the basis of biomarker measurements is not trivial, there has been considerable research in this area (for example, see Aylward, Hays *et al*., 2010; Hays *et al*., 2008; Krishnan *et al*., 2010a, 2010b). Indeed, cholinesterase inhibition has been used as a component of medical monitoring of exposed workers (reviewed in Garabrant *et al*., 2009) and could potentially be used in post-market surveillance.[101]

The most common pesticide-related biomarkers of effect have focused on the inhibition of acetyl cholinesterase, which is a marker of over-exposure to organophosphates and carbamate pesticides (ATSDR, 1997). Unlike biomarkers of exposure, measuring acetyl cholinesterase inhibition does permit consideration of variation in individual susceptibilities; however, interpreting the data may require baseline measurements and knowledge of time-of-exposure (Leng & Lewalter, 1999).

The Panel anticipates that identifying toxicologically relevant cellular response pathways will greatly enhance the identification of biomarkers of exposure and effect. This, in turn, will facilitate the early identification of effects in exposed populations. This is particularly relevant to the development of quantitative post-market surveillance studies. Biomarkers of exposure may include known metabolites of the chemical of interest, so long as they are relevant to the exposure scenario in question. Knowledge of the toxicokinetic properties of the agent (although almost never available for humans exposed to pesticides) is particularly useful in order to identify the most appropriate metabolite, tissue, and sampling approach (Benford *et al*., 2000). Biomarkers of effect are often far less specific to the agent in question and may be influenced by other effects, including nutritional status and cumulative exposures. Biomarkers of effect may be useful in post-market surveillance if there is enough evidence linking an adverse outcome to a particular exposure; however, it is anticipated that identifying appropriate biomarkers of exposure will be the most useful in post-market surveillance for pesticides.

Genetic susceptibility is deemed to be one of the main determinants of disease, so consideration of parameters that influence genetic susceptibility would represent a significant advancement in the risk assessment process. Understanding the factors

101 For example, see: http://www.lni.wa.gov/Safety/Topics/AtoZ/Cholinesterase/Providers.asp

that influence genetic susceptibility offers the potential to identify and characterize subpopulations that might be at particular risk as a result of an exposure. The challenge will be to identify those genetic markers that are functional with respect to specific chemical classes (or pesticide active ingredients) and to incorporate this knowledge into toxicity testing strategies as well as pre-market exposure modelling.

3.4.3 Development of Integrated and Interactive Knowledgebases

Chemoinformatics is a crucial component in evolving the tools and data sets necessary for an IATA approach (Richard *et al.*, 2008). Toxicity databases based on standardized schema, developed in conjunction with subject matter experts and populated with extensive data extractions, are prerequisites to the maturation of predictive toxicology.

Much of the existing (legacy) information on the effects of chemicals is dispersed throughout a large number of agencies and is not in forms that are compatible with modern, computer-assisted analyses. Digitization of existing data is an important first step in establishing an accessible and commonly formatted source of toxicological data. Although the effective capture and representation of legacy data by a number of initiatives illustrates the utility of building the informatics infrastructure (Knudsen *et al.*, 2009; Martin, Judson *et al.*, 2009; Martin, Mendez *et al.*, 2009), more effort will be needed to incorporate a wider range of participants in order to gather as much information as possible. Similarly, common platforms will need to be developed to ensure the consistent and uniform distribution of data to all potential users.

Providing downloadable, structure-searchable, standardized files associated with toxicity data ensures that structural analogues can be identified and that divergent data sets — for example, those generated in HTS — can be compared. Combining HTS results with physical chemical properties can establish structure-bioactivity relationships (SBARs). Because SBARs incorporate both chemical and biological aspects, they produce more robust predictions.

Efforts are needed to ensure that various national and international databases are compatible and interoperable. Equally, standards of good computer practice will need to be agreed upon. (for example, see P. N. Judson, 2009). After these common databases are developed, resources will be needed to maintain them. To enable computational models based on human (as opposed to solely laboratory animal) data, results from systematic studies of chemical effects in humans will be needed. These data are routinely gathered in clinical trials of new drugs submitted to the US FDA. To facilitate the inclusion of these types of data, the Tox21 Consortium (Box 3.13) has recently expanded to include the US FDA (Tox21, 2010).

3.4.4 Modernization of Existing Laboratory Practices

Good cell culture practice (GCCP) principles were developed to promote the maintenance of high cell culture standards to ensure the reproducibility, relevance, and acceptance of *in vitro* toxicity tests (Coecke *et al.*, 2005). These guidelines will need to be updated periodically to consider the unique needs of emerging cell culture technologies (Bal-Price & Coecke, 2011; Coecke *et al.*, 2005).

Ideally, the biological responses elicited by cell cultures used in an assay should approximate those exhibited by the *in vivo* system. Cell growth, cell morphology, gene expression, and level of differentiation in 2-D cultures differ considerably from those in 3-D culture systems (Yamada & Cukierman, 2007), and this may result in different cytotoxicity (Barcellos-Hoff *et al.*, 2005; Santini *et al.*, 1999).[102] Cell-to-cell communication and cell-substratum interactions are critical for signal transduction; studies with nano-structure surfaces have shown that extracellular signals may differ with each substratum, thus leading to variation in gene expression and cell behaviour (Ruiz *et al.*, 2008). Interestingly, contrary to the majority of non-stem cells, which would die by anoikis (apoptosis triggered by loss of extracellular matrix), stem cells may survive in a non-surface adhering situation by adhering to each other. This results in the development of "organoids," where variation in the microenvironment triggers the differentiation of some selected stem cells, which in turn induce modifications within the organoids (Markert, 1983).[103] Such 3-D organoids may start to mimic some of the complex interactions that occur during embryogenesis and organogenesis. Studies designed to identify and develop conditions for normal 3-D interactions will likely play an important role in establishing standards for subsequent assay development.

Quality control criteria that can assess the process of reprogramming will be needed in order to ensure the biological relevance of the cell system for answering specific toxicological questions. These might include identifying stage-specific embryonic and lineage markers, expressed during differentiation. Other factors that need to be controlled include the growth phases of the cultures, the level of oxygen in the microenvironment, and the growth factors, nutrients, and trace elements used in the growth media. The maintenance of embryonic stem cells, mesenchymal stem cells, and specific organ adult stem cells *in vitro* all also require consideration of specific factors (Csete, 2005; Linning *et al.*, 2004).

102 2-D cell culture techniques typically use monolayer cultures grown in petri plates. 3-D cell culture techniques use methods that permit the aggregate growth of cells, which more closely mimics physiological conditions.

103 It is also possible to induce organoid formation with non-stem cell cultures, e.g., hepatocytes.

3.4.5 Validation and Acceptance of Alternative Test Methods

Before alternative testing strategies can be used in a regulatory context, their validity with respect to the toxicity endpoint they are designed to assess must be demonstrated. OECD specifically defines an endpoint as a "test protocol endpoint" (OECD, 2007b). This implies that any validated test (or battery of tests) must be developed and validated as a one-for-one replacement of an existing protocol.

Alternative methods (either testing or non-testing) typically target specific cellular or physiological responses and, as such, preclude validation with *in vivo* data by a one-for-one approach. The AOP allows for the use of a suite of assays (and subsequent databases) that are designed to target particular steps along a specific pathway. Each assay/data set in a suite of information would inform the next tier of the IATA or be used as part of overall Integrated Testing Strategies (ITS). The scientific justification of an alternative method or data set should focus on comparing the test outcome to what is known about the underlying biology as described in the AOP, and thus aid in the decision-making process. Not all the key events in an AOP, all the tiers in an IATA, or all the aspects of an ITS have to be satisfied to make an assessment. Furthermore, once a suite of predictive assays has been developed and shown to be reliable, industry could use them to help provide definitive information on the safety of compounds early in the product development process. This would be of significant benefit to industry and might help expedite the time to market for safer chemical products.

Validating an alternative test method against the data produced by an *in vivo* assay (that itself may not have been validated) speaks to a philosophical flaw in the current approach to scientific validation. The Panel believes that it is time to move away from thinking about validation as a one-for-one replacement of an existing animal study and towards a new approach that is anchored in understanding the underlying biology. This will be discussed in more detail in Chapter 4.

3.5 TRANSITIONING TO THE FUTURE

As HTS, computational toxicology, systems biology, bioinformatics, and other IATA tools move from concept to application, a number of national and international efforts to integrate data are underway. These efforts include the development of *in vitro* test methods, high-throughput *in vitro* tests and test batteries; the development of *in vitro* signatures of bioactivity based on aggregate HTS data; integration of results from omics studies, especially toxicogenomics; and the

development of software to facilitate chemical categorization to aid (Q)SAR models. The common denominator to all these activities is a movement away from phenomenological observation and towards an understanding of mechanisms of toxic action as the foundation for toxicological evaluation.

While these efforts are in their infancy, and the specifics vary, their central objective is to generate and integrate data from *in vitro*, *in vivo*, *in chemico*, and *in silico* sources in much better ways. The shorter-term goal is to link *in vitro*, in chemico, and *in silico* data to *in vivo* outcomes derived from standard test guidelines in mechanistically plausible schemes. Over the long term, the intent is to test tens of thousands of chemicals with rapid and inexpensive molecular screening techniques, verify their biological activities by testing hundreds of chemicals in more physiologically complex *in vitro* tests, and thus test only tens of chemicals *in vivo*. The ultimate goal is to prospectively and accurately assess chemical hazards with methods that use fewer animals and fewer resources in order to focus finite resources on those chemicals and endpoints of greatest concern.

Chapter 3 has addressed the state of the science of IATA-related tools; however, the science alone provides only the evidence base to inform to regulatory decision-making. Efforts in science must proceed with a functional collaboration between scientists and regulators in order to ensure that the science evolves into a form that is applicable to regulatory use. Chapter 4 will evaluate the state of use of IATA tools and address the barriers and opportunities relevant to IATA in a regulatory context.

3.6 CHAPTER SUMMARY

What is the state of the science of the tools and data sources associated with integrated testing strategies?

Integrated Approaches to Testing and Assessment (IATA) represent a pragmatic approach that will move toxicology away from describing *what* happens towards explaining *how* it happens. There is no single IATA however. Fundamental to the use of any IATA is the existence of an adverse outcome pathway (AOP) that causally relates key events at different levels of biological organization to the *in vivo* endpoint of regulatory interest.

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CHAPTER SUMMARY *(continued)*

IATA can adopt and integrate tools from a wide variety of disciplines; these tools are all at different stages of readiness and are continuously evolving. Some use computational approaches to leverage existing toxicity data; others focus on the generation of new data. At the heart of this evolution are the fields of systems biology and computational toxicology.

Systems biology incorporates data from a wide range of disciplines. It relies on existing data from *in vivo* studies and new knowledge from cell biology and omics research. It also relies on bioinformatics and computational biology to provide the platforms necessary to collate, sort, and search the vast quantities of data that must be considered in developing a systems-level understanding of a complex biological system. Systems biology provides the basis for understanding mode of action (MoA) and then identifying thresholds for the perturbation of critical cellular pathways; these thresholds will form the basis for the development of quantitative HTS assays as well as for identifying biomarkers of exposure and effect.

Computational toxicology permits the categorization chemicals based on their inherent properties in order to screen and prioritize them for further toxicity testing. It does this by using relational databases that are able to cross-reference existing data from a multitude of sources. Computational toxicology also harnesses the advances made in systems biology to develop models of predictive toxicity that are anchored in a mechanistic understanding of human physiology. Computational toxicology is only as good as the data it is built on, so international efforts to digitize existing toxicity data and develop common ontologies are crucial to strengthening its utility and power. Since the vast majority of chemicals are currently data-poor, computational approaches still have limited predictive ability. However, with the generation of large quantities of *in vitro* bioactivity data on thousands of chemicals via HTS approaches, computational approaches are evolving rapidly. Open-access, relational databases and knowledgebases that can cross-reference *in vivo* and *in vitro* data combined with common ontologies that can account for historical differences in vocabulary and nomenclature will become increasingly important.

Although IATA can fill data gaps for data-poor compounds, the chemical and biological understanding of AOPs or MoAs could also be used to assess data-rich chemicals, e.g., pesticide active ingredients. Specifically, developing a better toxicological understanding could help to streamline the testing and assessment of new pesticide active ingredients.

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CHAPTER SUMMARY *(continued)*

Once an AOP/MoA has been established, the key events data could be used for read-across from other chemicals. If a new pesticide fits a previously established AOP, this existing knowledge would then be used to justify a more efficient testing strategy so that not every endpoint will need to be evaluated in an expensive *in vivo* test. (The Panel acknowledges that this does not eliminate the possibility of more than one MoA or AOP; therefore, the development of a library of AOP/MoAs would fully realize the utility of this approach).

The acceptability and applicability of any new tools for use in a regulatory context will be enhanced by the engagement of the international regulatory community and the execution of proof-of-concept studies that build confidence and familiarity in new approaches. Indeed, over the past five years, significant research efforts have been focused on developing new approaches and models for predictive toxicology and executing robust, proof-of-concept studies. As a result of these studies, IATA tools can be used to predict some acute toxic endpoints, such as skin irritation. In the short term (next one to two years), additional IATA approaches to evaluate critical local effects will likely be available.

Although non-animal replacement approaches for complex endpoints (e.g., carcinogenicity, reproductive toxicity) are more challenging, high-throughput screening (HTS) assays are currently generating data on thousands of chemicals. While it could be at least a decade before they are ready to be used in a regulatory context for data-rich chemicals, they may prove useful in filling information gaps for data-poor chemicals in the interim.

Epidemiological studies characterize real-life exposure scenarios of a chemical agent, including population-level effects, which could be tremendously valuable in identifying unanticipated health outcomes during the post-market phase. Identifying and adopting appropriate biomarkers of exposure and disease would facilitate the practical deployment of these kinds of studies, which would strengthen the regulatory process and increase the depth and robustness of existing epidemiological data sets. This would, in turn, permit using existing epidemiological data in pre-market exposure modelling and assessment of related chemicals.

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CHAPTER SUMMARY *(continued)*

Alternative methods (either testing or non-testing) typically target specific cellular or physiological responses and, as such, preclude validation with *in vivo* data by a one-for-one approach. The AOP allows for the use of a suite of assays (and subsequent databases) that are designed to target particular steps along a specific pathway. Each assay/data set in a suite of information would inform the next tier of the IATA or be used as part of an overall ITS. The scientific justification of an alternative method or data set should focus on comparing the test outcome to what is known about the underlying biology as described in the AOP and thus aid in the decision-making process. Not all key events in an AOP, all tiers in an IATA, or all aspects of an ITS, may have to be satisfied to make an assessment. Furthermore, once a suite of predictive assays has been developed and shown to be reliable, industry could use them to help provide definitive information on the safety of compounds early in the development process. This would be of significant benefit to industry and might help expedite the time to market for safer chemical products.

4

The Status of the Use of Integrated Testing Strategies for Risk Assessment

- Current Applications of IATA in Canada, the United States, and Europe
- Scientific Validation and Regulatory Acceptance of IATA Tests
- Addressing the Needs of Regulators and the Regulatory Process: The Need for Functional Engagement

4 The Status of the Use of Integrated Testing Strategies for Risk Assessment

What is the Current Status of the Use of Integrated Testing Strategies for the Risk Assessment of Pesticides, Pharmaceuticals, Industrial Chemicals, and Other Chemical Substances by Regulatory Agencies around the World?

LIST OF KEY TERMS*
Cytotoxicity:
The degree to which an agent causes damage to cell structure or function.
Endocrine Disruptor:
An exogenous substance that can change endocrine function and cause (potentially adverse) effects at the level of the organism, its progeny, and/or (sub)populations of organisms.
Margin of Safety:
The margin between the reference dose (RfD) and the actual exposure dose or concentration.
Reverse Pharmacokinetics:
An approach that extrapolates from an effective *in vitro* concentration to an equivalent human exposure level (or dose).
Toxicity Screen:
An experimental approach designed to generate specific toxicity data on a chemical in order to characterize its intrinsic toxicological properties.
Validation:
The process of testing the reliability and relevance of a test method. Reliability considers the reproducibility of test results. Relevance describes the usefulness of the data produced for their intended purpose.

*Key terms as used by the Panel throughout this report. Additional terms are listed in the Technical Glossary in Appendix A.

4.1 CURRENT APPLICATIONS OF IATA IN CANADA, THE UNITED STATES, AND EUROPE

As discussed in Chapter 3, an IATA approach seeks to integrate all useful data — including chemical categorization and prioritization — to inform a risk assessment via a hierarchical approach to testing (Figure 3.1). This holistic approach can be extremely powerful in a regulatory context. It can expedite the assessment process for low-risk chemicals, ensure that higher-risk chemicals are flagged for additional testing earlier in the process, and increase the overall number of chemicals that can be evaluated.

This chapter will highlight examples where an IATA strategy (or components of an integrated approach) has been used to support regulatory decision-making for chemicals in general, and specifically pesticides, where applicable. It will also identify what kinds of alternative tests were used (i.e., testing versus non-testing tools) and for what purposes. In order to contain the discussion, the Panel focused its attention on regulatory implementation in Canada, the United States, and the European Union.

This chapter will conclude by discussing challenges and opportunities for regulatory reform. These challenges and opportunities are based on the Panel's belief that regulatory decisions should be founded on the best available science; any changes in the regulatory toxicity testing paradigm should be driven by this guiding principle.

The active ingredients of pesticides are one of the most stringently regulated groups used in commerce; the toxicological assessment of the active ingredient follows a regimen that is similar to that for the preclinical assessment for the safety of a prescription drug. This makes pesticide active ingredients one of the most data-rich groups of chemicals in commerce (as discussed in Chapter 2). This is quite different from the data-poor nature of many other chemicals, including most industrial chemicals, which have historically not been subject to extensive pre-market toxicity testing. Some countries have taken steps to address the lack of toxicity data on these chemicals, particularly those that are either manufactured or imported in large quantities — the so-called high production volume (HPV) chemicals.[104] The sheer number of these agents precludes a comprehensive toxicity assessment using conventional means; therefore, measures to address the data gaps must rely on alternative approaches to identify chemicals whose toxicity profile warrants further investigation.

104 The OECD defines HPV chemicals as those that are produced at levels greater than 1,000 tonnes per year in at least one country or region (OECD, 2004a).

4.1.1 Canada

Canada is a world leader in the development and implementation of *in silico* screening and prioritization tools. This has been primarily driven by the *Canadian Environmental Protection Act* (CEPA). This Act provided the legislative mandate to categorize and establish assessment priorities for the approximately 23,000 substances on the Domestic Substances List (DSL) by September 2006 (Government of Canada, 1999) and led to the development of the Chemicals Management Plan (CMP).[105] The objective of the CMP is to assess all DSL chemicals in Canada by 2020 and to develop risk management strategies where necessary. The CEPA and CMP will be discussed in more detail in the following subsections.

The *Canadian Environmental Protection Act* (1999):

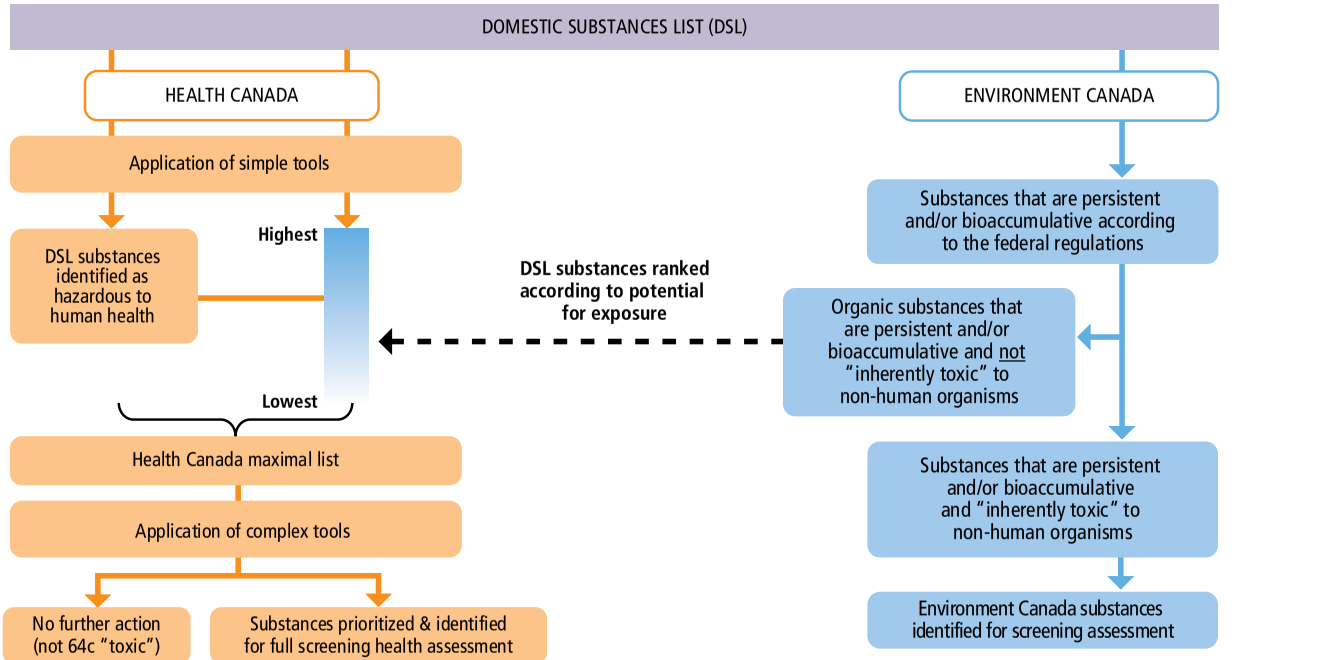
Since 1 July 1994, anyone wishing to import or manufacture a new substance in Canada must submit notification to the New Substances Program (Government of Canada, 1994b, 2005).[106] The approximately 5,000 transitional substances that were first introduced into Canadian commerce between 1987 and 1994 were assessed for health hazard and predicted exposure (Environment Canada, 1994); however, chemicals introduced prior to this were not. The DSL is an inventory of the approximately 23,000 substances that were manufactured in, imported into, or used in Canada between 1 January 1984 and 31 December 1986 (Environment Canada, 2010).

CEPA, 1999 directed the Minister of the Environment and the Minister of Health to identify and categorize those chemicals on the DSL that are persistent or bioaccumulative and inherently toxic to humans or that pose the greatest potential for human exposure.[107] These statutory requirements under CEPA required coordination between federal agencies as well as the development of novel predictive tools that could help set priorities based on exposure and hazard information (Figure 4.1) (Patterson *et al.*, 2007). The first tier of this screening process uses conservative, predictive modelling and assimilates structural data and existing toxicity information to aid in hazard identification.

105 The DSL is also referred to as the Existing Substances List.

106 A New Substance is broadly defined as any substance not listed on the DSL.

107 This necessitated a careful consideration of what "toxic" meant under CEPA. Under CEPA, both the hazard and exposure are considered when assessing the toxicity of a substance (Environment Canada, 2006; Health Canada, 2007).



(Reproduced with permission from Health Canada)*

Figure 4.1

Screening of the 23,000 substances on the DSL required coordination between federal agencies as well as the development and use of specialized predictive tools

*The Health-Related Components of Categorization of the Domestic Substances List (DSL): Approach, Results, and Next Steps Health Canada, 2008 reproduced with the permission of the Minister of Health, 2011.

Health Canada developed two prioritization tools in order to complete a comprehensive evaluation of existing data. These tools were used to identify data gaps and prioritize chemicals for further testing. They made use of existing data in a hierarchical fashion that considered multiple endpoints of concern and potential health impacts (described in more detail in Box 4.1).

Box 4.1
CASE STUDY: Prioritization Tools for Hazard Prediction under CEPA

SimHaz was designed to screen and prioritize substances by comparing them with lists of high- and low-hazard substances developed by other agencies (reviewed in Hughes *et al*., 2009).[108] However, SimHaz was less useful as a tool for identifying substances that needed further data because it drew heavily on data from existing prioritization initiatives and tended to be biased towards the data-rich compounds (Hughes *et al*., 2009).

ComHaz, a more complex hazard tool, permits a systematic, hierarchical analysis of multiple endpoints based on potential health impacts as well as regulatory reference values (Figure 4.2). Non-threshold chemicals are considered before threshold ones. If a chemical meets the criteria associated with a given endpoint it is prioritized for further assessment, which includes consideration of dose-response effects (Hughes *et al*., 2009).

The sources of information used were also prioritized in descending order of confidence from primary toxicity data down to non-quantitative SARs (Figure 4.3). All data were also subject to expert scientific judgment, independent of their original source.

108 These lists were evaluated for robustness and transparency. A full list of lists may be found in Hughes *et al*. (2009).

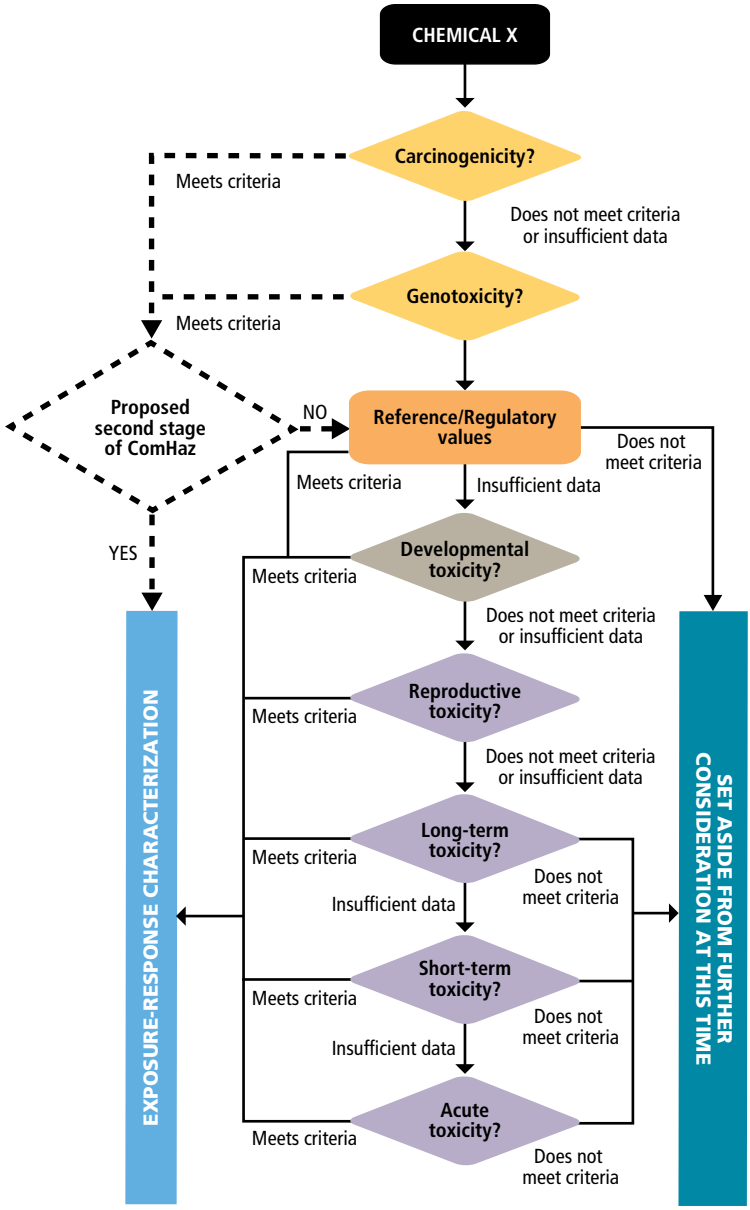


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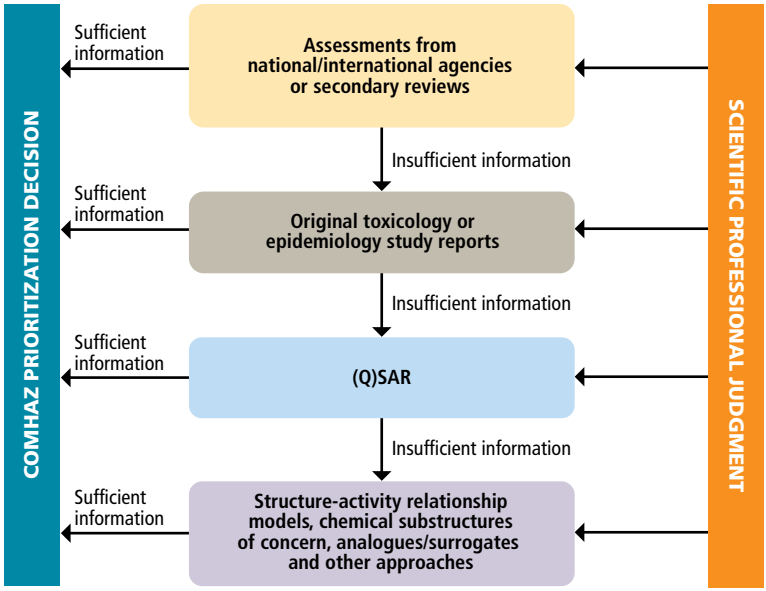
Figure 4.2

ComHaz permits the hierarchical prioritization of chemicals

*Reproduced from: *Regulatory Toxicology and Pharmacology*, 55/3, K. Hughes, J. Paterson, M.E. Meek, Tools for the prioritization of substances on the Domestic Substances List in Canada on the basis of hazard, 382-393, Copyright (2009), with permission from Elsevier.



(Reproduced with permission from *Regulatory Toxicology and Pharmacology*)*

Figure 4.3
Hierarchical prioritization of data sources by ComHaz

*Reproduced from: *Regulatory Toxicology and Pharmacology*, 55/3, K. Hughes, J. Paterson, M.E. Meek, Tools for the prioritization of substances on the Domestic Substances List in Canada on the basis of hazard, 382-393, Copyright (2009), with permission from Elsevier.

The screening and prioritization was completed in 2006, making Canada the first country to systematically evaluate all chemicals currently in commercial use (reviewed in UN-DSD, 2009). Of the 23,000 chemicals assessed, 4,300 were prioritized for further testing under Canada's Chemicals Management Plan (CMP) and 500 of these were classified as being of highest priority.[109]

The CMP was introduced in 2006 under the jurisdiction of CEPA in order to further evaluate those chemicals identified as being of highest priority. The fundamental goal of the CMP is to use proactive measures that will ultimately improve well-being; reduce the costs of environmental clean-up; help to establish Canada as a world leader in science-based policy; and improve the conditions for businesses in Canada (reviewed in Briand, 2010).

109 For more information, see http://www.parl.gc.ca/HousePublications/Publication.aspx?DocId=3077462&Language=E&Mode=1&Parl=39&Ses=1

Of the 500 highest priority chemicals identified in the CEPA-mandated screening process, approximately 140 were in commercial use in Canada at the time of evaluation; 160 have been grouped for assessment by the petroleum industry; and 200 are under evaluation via the Industry Challenge program. The Industry Challenge program requires industry to provide specific information (in the form of a survey) to facilitate the drafting of a screening assessment that will be used to determine whether a chemical warrants additional risk management. Under the Industry Challenge program, importers, manufacturers, and users are required to provide governmental regulators with data concerning usage and exposure.

Once this process has been completed for the high-priority chemicals, attention will shift towards those designated as medium and low priority. Consultations are also being conducted to determine how to meet the goal of assessing and managing these substances by 2020.

4.1.2 European Union

The European Union (EU) is cited as a world-leader in the promotion and development of alternative testing strategies (Hartung, 2010). Two major European initiatives — Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and the Cosmetics Directive — are likely to result in consequences that will extend far beyond EU borders, with significant impacts on international industry.[110]

REACH:

REACH came into effect on 1 June 2007 to address the lack of available safety information on the majority of chemicals in wide use. A further aim is to eliminate redundancy, inefficiency, and ineffectiveness by replacing approximately 40 separate pieces of legislation with a single approach to chemical regulation (reviewed in European Commission, 2007).[111] Under the previous legislation, "new" and "existing" chemicals were regulated differently. New substances introduced after 1981 were subject to some pre-market toxicity testing; however, existing chemicals — defined as those on the market between 1 January 1971 and 18 September 1981 and listed on the European Inventory of Existing Commercial Chemical Substances (EINECS) — were "grandfathered in" (European Commission, 1998; and reviewed in Williams *et al.*, 2009). As a result, although some toxicity data were available for approximately 65 per cent of HPV chemicals, it was less

110 The Cosmetic Directive refers to Directive EC 76/768/EEC that was revised on January 2003 to ban 1,100 chemicals from cosmetics. Regulation (EC) No 1223/2009 was subsequently adopted on 30 November 2009. Most of the provisions of this new regulation will be applicable as of 11 July 2013 and will replace the Cosmetics Directive (76/768/EEC).

111 Although REACH does not specifically apply to pesticide active ingredients, it does apply to formulants and non-pesticidal uses of active ingredients (Bergeson *et al.*, 2008).

than the base set of data required for new chemicals; it was estimated that 21 per cent of chemicals had no assembled data at all (Allanou *et al.*, 1999).[112] (Although a detailed discussion of the REACH process is beyond the scope of this report, a brief overview is provided in Box 4.2.)

Box 4.2
REACH in Brief

REACH is the Registration, Evaluation, Authorisation and Restriction of Chemicals legislation that governs industrial chemicals in the EU.

Registration

Under REACH, all manufacturers, importers, or users of more than one tonne of qualifying substances per year must register the substance with the European Chemicals Agency (ECHA). To do so, they must provide the following:

- A technical dossier for substances of one tonne or greater; and
- A chemical safety report (CSR) for substances of 10 tonnes of greater.

The extent and nature of toxicological data that must be included in the technical dossier, and CSR, increase with increasing volume, with tonnage essentially acting as a surrogate for predicted human and environmental exposure (Williams *et al.*, 2009).[113]

The information requirements for registration under REACH grow with increased volume

Tons/ year	Required Information on Intrinsic Properties					
	PC, toxicity and ecotoxicity information	PC properties	Toxicity and ecotoxicity information		PC, toxicity and ecotoxicity information	Toxicity and ecotoxicity information
	All available relevant data	Annexe VII requirements	Annexe VII requirements	Annexe VIII requirements	Annexe IX requirements	Annexe X requirements
1–10	X	X	X			
10–100	X	X	X	X		
100–1,000	X	X	X	X	X	
>1,000	X	X	X	X	X	X

(Adapted from CNRS, 2007)

continued on next page

112 10,000 substances were sold in annual volumes greater than 10 tonnes, and 20,000 substances were sold in quantities of 1–10 tonnes.

113 Specific guidance on these data requirements is provided in Annexes VII through XI of the REACH Regulation (European Union, 2006).

Box 4.2 *(continued)*

A CSR is required for all substances that are manufactured, imported, or used in excess of 10 tonnes per year. The CSR includes specific risk assessments — also referred to as Chemical Safety Assessments (CSAs) — for all identified uses of the substance in question (Schoeters, 2010).

The registration process for existing chemicals is being phased in from November 2010 until May 2018. By June 2018, dossiers describing the physicochemical, toxicological, and ecotoxicological properties of all chemicals marketed in amounts that exceed one tonne per year per company must be complete (Schoeters, 2010).

Evaluation

Dossier evaluation for testing proposals to fill data gaps will be mandatory for all substances that fall in the two largest tonnage classes (reviewed in Warhurst, 2006). Approximately five per cent of the dossiers submitted for the lower tonnage classes will be assessed by ECHA to ensure compliance (Williams *et al*., 2009).

Authorization

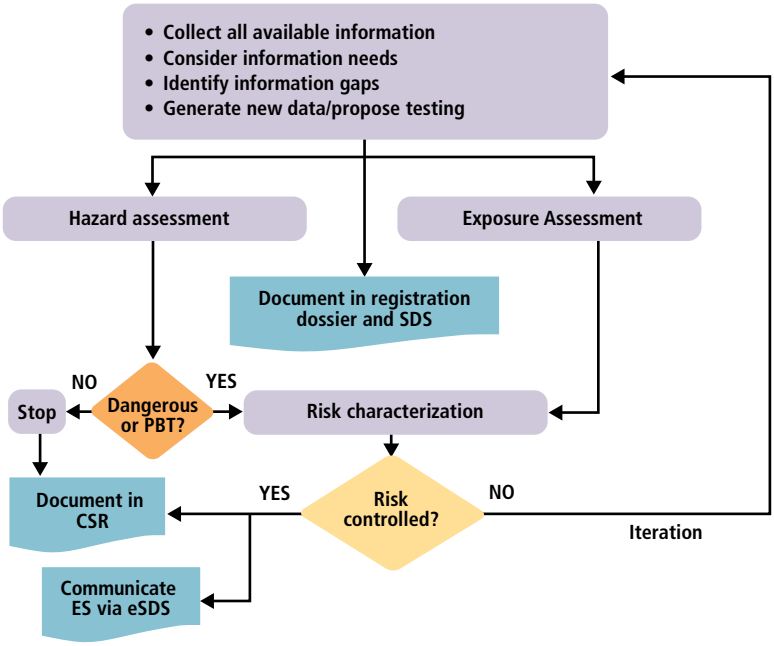
The purpose of authorization is to control the use of those chemicals of high concern. These include substances that are:

- classified as carcinogens, mutagens, or reproductive toxicants;
- persistent, bioaccumulative, or toxic (PBT);
- very persistent and very bioaccumulative (vPvB); or
- cause an equivalent level of concern on the basis of probable serious effect to human health or the environment.

Authorized chemicals will be subject to periodic reviews, and their use will be limited solely to those specified in the authorization application. Furthermore, applications for authorization will be made public, to promote the development and adoption of safer alternatives.

Restriction of Chemicals

Member states may seek to restrict the manufacture, sale, or use of substances deemed to pose "unacceptable risks to human health and the environment." Chemicals do not necessarily need to be registered in order for a member state to initiate a restriction application, which provides a mechanism for dealing with chemicals that are exempt from registration, or existing chemicals that have not yet been registered (Warhurst, 2006).



(Reproduced with permission from European Chemicals Agency, http://echa.europa.eu/)

Figure 4.4

The REACH process

By explicitly mandating the use of all available data to inform a risk assessment, REACH is the first tangible example of a legislative initiative that embodies IATA.

Although actual estimates vary, meeting the data requirements of REACH using conventional *in vivo* toxicity tests would be expensive and would necessitate the use of large numbers of animals. The EU initially estimated €1.2–€2.4 billion and 2.1–3.9 million animals; this estimate was challenged by Rovida and Hartung (2009) who predicted the costs would be closer to €9.5 billion and 54 million animals, while others estimate that as many as 141 million animals might be needed (Schoeters, 2010).[114] Regardless of the final numbers, REACH's mandate is unlikely to be accomplished without significant costs or the adoption of alternative toxicity testing approaches.

114 These studies claim to account for factors not included in the initial estimates: EU expansion from 12 to 27 members, plus three non-EU countries that will follow REACH; the almost two-fold growth of the European chemical industry between 1994 and 2008; and the expected increase in use of a costly two-generation reproductive toxicity studies, resulting in 3,200 rats per chemical rather than the initial estimate of 784 for a one-generation study (Hartung & Rovida, 2009; Rovida & Hartung, 2009).

Indeed, REACH explicitly states that any new vertebrate studies are to be performed only as a last resort. To this end, the legislation specifically calls for adopting IATA strategies including the optimal use of existing data, the elimination of duplicate toxicity studies, and the promotion of data sharing (European Union, 2006). It also prescribes a number of options to ensure that all existing (and scientifically credible) data are used in the screening and prioritization process (European Union, 2006):

- Consideration of existing toxicity and ecotoxicity information before making decisions about subsequent data requirements. This information can include epidemiological studies, computer models, and international data sources.
- The use of alternative testing methods, including chemical categorization, in order to minimize the testing burden.
- The development of tailor-made testing programs for HPV chemicals under the control of authorities.
- A duty to inquire about prior registration of the substance in order to avoid the repetition of vertebrate studies.
- The establishment of a Substance Information Exchange Forum (SIEF) to facilitate the sharing of data on a particular substance between registrants, in order to avoid duplication of animal studies.

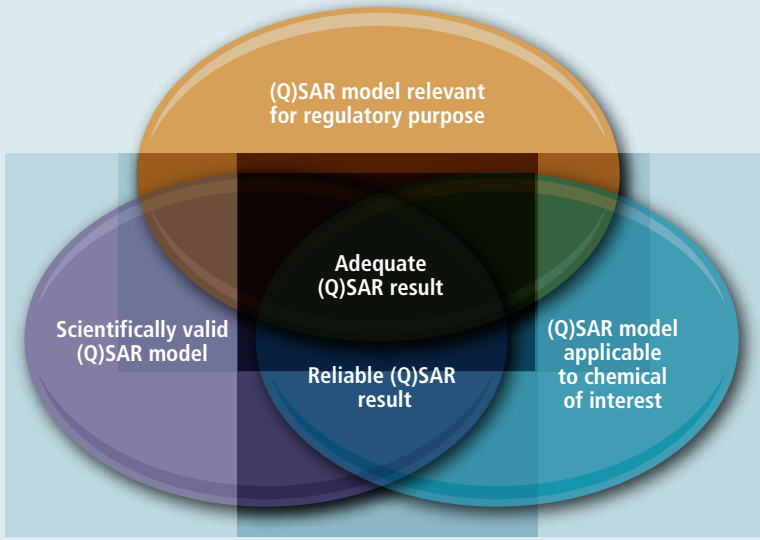
Furthermore, there have been a number of articles on and proposals for how integrated approaches may be used in the context of REACH (for example, see the following reviews: Ahlers *et al.*, 2008; de Wolf *et al.*, 2007; Grindon *et al.*, 2006; Grindon *et al.*, 2008a, 2008b, 2008c, 2008d, 2008e; Gubbels-van Hal *et al.*, 2005; Hoffmann *et al.*, 2008). Where data gaps exist, REACH uses (Q)SAR results for endpoints so long as chemicals and endpoints in question fall within the applicability domain of the model and the model itself has been appropriately validated (Box 4.3) (reviewed in Williams *et al.*, 2009). It also permits using data from *in vitro* studies, providing they are derived from a validated testing method; however, the number of validated tests relevant to REACH is currently quite small.

Approximately five times the number of existing test guidelines may be required to accommodate what is proposed under REACH (Hartung, 2009; Hartung & Rovida, 2009); this will necessitate a considerable refinement of the formal test validation process in order to ensure that REACH testing needs can be met. It is therefore anticipated that REACH may be a driver of both scientific change and expedite regulatory acceptance of alternative testing methods.

Box 4.3
CASE STUDY: Use of (Q)SAR Under REACH

REACH outlines a framework that explicitly permits the use of (Q)SAR-derived data in place of experimental data if four primary conditions are met:

- the scientific validity of the model has been established;
- the applicability of the model to the chemical of interest has been demonstrated;
- the relevance of the prediction (result) is appropriate for the regulatory purpose in question; and
- adequate and reliable documentation for the method and results is provided.



(Reproduced with permission from Springer Science and Business Media)*

Usage of (Q)SAR models under REACH

*Reproduced with permission from Springer Science+Business Media: Recent Advances in QSAR Studies: Methods and Applications (Vol. 8), The Role of QSAR Methodology in the Regulatory Assessment of Chemicals, 8, 2010, Worth, A. P., pg. 371, Figure 13-1.

The EU Cosmetics Directive and Product Regulation:

The EU Cosmetics Directive was enacted in 1976 to ensure the appropriate management and safe use of cosmetic products in Europe (European Union, 1976). The initial directive defined "cosmetic" and listed substances that could not

be used, or only in specified circumstances. It also outlined labelling requirements that mandate full disclosure of ingredients, expiration dates, intended use, and necessary precautions. The Cosmetics Directive has since been amended seven times, to reflect the changing landscapes of both cosmetics marketing and safety testing.

The Sixth Amendment of the EU Cosmetics Directive (European Union, 1993) introduced a marketing ban on products that had been tested on animals. This was revised in 2004 by the Seventh Amendment, which banned the testing of finished cosmetic products on animals on 11 September 2004 and of ingredients (or combinations of ingredients) by 11 March 2009 (European Union, 2009e). The Seventh Amendment also introduced a marketing ban for all human health effects — with the exception of repeat-dose toxicity, reproductive toxicity, and toxicokinetics — from 11 March 2009. The Amendment explicitly stated that this ban would be upheld regardless of the availability of validated alternative test methods. A marketing ban for all tests (including repeat-dose toxicity, reproductive toxicity, and toxicokinetics) was set for 11 March 2013 (European Commission, 2004); however, the possibility of a postponement of this deadline was explicitly mentioned (Rossignol, 2005). On 30 November 2009, under the EU regulation 1223/2009, the European Cosmetics Directive was replaced by the European Cosmetic Products Regulation (European Union, 2009a), making the ban on animal testing for cosmetic products legally and directly binding in all member countries.[115]

The endpoints affected by the EU Cosmetics Regulation, the cut-off dates for their replacement, and the status of validated alternative tests are summarized in Table 4.1. Alternative tests were available for all but two of the endpoints affected by the March 2009 cut-off; for these remaining two endpoints, industry has been allowed to rely on data from tests produced before March 2009 (European Commission, 1991). Considerable work is still required to meet the needs of endpoints covered by the March 2013 deadline. These endpoints are toxicologically more complex, which makes the development of valid alternatives considerably more complicated. The European Commission is required to inform the European Parliament and Council in 2011 of the status of alternative tests covered by the 2013 deadline (European Commission, 2008). If appropriate, a legislative proposal to extend the deadline will be presented. While this report was in development, a series of expert reviews to inform this proposal were available in draft form (Adler *et al.*, 2010; Basketer *et al.*, 2010; Creton *et al.*, 2010; European Commission, 2010; Pelkonen *et al.*, 2010; van Benthem *et al.*, 2010). Final reports were scheduled to come out at the end of 2011.

115 Implementation of directives is the responsibility of each member country. In contrast, regulations are immediate and legally binding in every member country; they require no action from the national authorities.

Table 4.1

Safety endpoints affected by EU Cosmetics Regulation and the timetable for phasing-out of animal tests

Endpoint	Cut-Off Date	Status at Time of Cut-Off
Acute toxicity	11 March 2009	Not met
Skin irritation	11 March 2009	Met
Eye irritation	11 March 2009	Not met
Skin sensitization	11 March 2013	n/a
Skin penetration	11 March 2009	Met
Sub-acute/chronic toxicity	11 March 2013	n/a
Genotoxicity	11 March 2009	Met
UV-Induced toxicity	11 March 2009	Met
Photogenotoxicity	11 March 2013	n/a
Photoallergy		
TK and metabolism	11 March 2013	n/a
Carcinogenicity	11 March 2013	n/a
Reproductive & developmental toxicity	11 March 2013	n/a

(Adapted from RSC, 2006)

Box 4.4
An Aside on the Regulation of Personal Care Products in Canada

In Canada, cosmetics are regulated by the Healthy Environments and Consumer Safety Branch (HECSB) of Health Canada under the Cosmetics Regulations of the *Food and Drugs Act* (Government of Canada, 1985c).[116] Under this Act:

- Cosmetics manufacturers must disclose product ingredients to Health Canada.
- Health Canada reviews the list of ingredients for substances included on the Cosmetics Hotlist.[117]
- Health Canada product safety inspectors investigate consumer complaints or reports of adverse reactions and can require a product be withdrawn if they determine that it is unsafe.
- Cosmetics companies must list the ingredients of their products on the label in order to sell it in Canada.

116 Note that new and existing cosmetics ingredients are also governed by CEPA.

117 Cosmetics Hotlist: http://www.hc-sc.gc.ca/cps-spc/person/cosmet/info-ind-prof/_hot-list-critique/hotlist-liste-eng.php

Neither Canadian nor U.S. law explicitly require (or prohibit) the testing of cosmetic ingredients or products on animals; however, they explicitly require that the product not cause damage to human health when used in accordance with manufacturer's instructions (Box 4.4).[118]

In keeping with other regulated chemicals, the burden of safety evaluation for cosmetics lies with the manufacturer; however, there is one major difference that must be considered in drawing parallels between the regulatory deployment of alternative tests for cosmetics and other groups of data-rich chemicals. In contrast to pharmaceuticals and pesticides, a personal care product is not subject to a comprehensive risk assessment evaluation by government regulators (Rogiers & Pauwels, 2008).

4.1.3 The United States

In contrast to the European approach, regulatory developments in the United States have often been driven by an agency vision (Hartung, 2010). The Panel has chosen to highlight two of these here: the *Toxic Substances Control Act* (TSCA) and the Endocrine Disruptor Screening Program (EDSP). In addition, the Panel will illustrate a potentially novel use for high-throughput assay systems in a regulatory context. Following the explosion on the Deepwater Horizon oil platform, the US EPA used their ToxCast™ program (Box 3.15) to facilitate the rapid screening of oil dispersants for a number of toxicity endpoints. This permitted regulators to make science-based decisions in an emergency in a way that would not have otherwise been possible.

Toxic Substances Control Act:

Under the Premanufacturer Notification (PMN) requirements of the TSCA, the US EPA must evaluate and make predictions about the chemical identity, physical/chemical properties, environmental transport and partitioning, environmental fate, environmental toxicity, engineering releases to the environment, and environmental concentrations of a chemical (United States Government, 1976). The US EPA is authorized to require additional testing if certain exposure or volume triggers are met (reviewed in Wagner *et al.*, 1995). Under TSCA, manufacturers are not required to conduct toxicity testing on a new chemical prior to submission of a PMN; however, any data that they do possess must be provided at the time of submission. Typically, some acute and/or mutagenicity data are submitted on approximately 40 per cent of chemicals (Wagner *et al.*, 1995). TSCA requires the

118 Under the original cosmetics legislation, animal testing was not explicitly required; however, the state of the science of toxicity testing precluded the development of adequate safety data without the use of animal tests.

US EPA to evaluate available data and conduct a preliminary hazard assessment within 90 days of receipt of a PMN. Given the data-poor nature of the chemicals under review, meeting this legislative mandate necessitated adopting screening and prioritization tools. A number of tools were subsequently developed, including Estimation Program Interface (EPI) Suite, Ecological Structure Activity Relationships (ECOSAR), and OncoLogic (reviewed in US EPA, 2010m).

The US EPA has routinely integrated (Q)SAR modelling, chemical categorization/read-across methods, and any existing information for both toxicity and exposure to help determine potential human health and ecological hazard when data are lacking, particularly for new and HPV chemicals (reviewed in Richard, 1998; US EPA, 2010h, 2010i, 2010l). A chemical that is identified as a potential health hazard may either be required to undergo further testing or be removed from production (Richard, 1998).

TSCA reform has been debated considerably in recent years (for example, see Locke & Myers, 2010), and bills to reform TSCA have been introduced in the United States Senate and House of Representatives (United States Congress, 2010; United States Senate, 2010). If such bills are passed they will require manufacturers to provide and the US EPA to assess data on all of the chemicals currently in commercial use. This mandate could not be met using the existing approach to toxicity testing, and specific provisions are being made for the development of alternative approaches. In addition, these bills would give the US EPA the authority to modify the testing requirements for a given chemical based on existing data, facilitating a transition towards an approach in which testing resources are targeted to the endpoints of highest concern.

The US EPA Endocrine Disruptor Screening Program:

Following passage of the *Food Quality Protection Act* of 1996 (United States Government, 1996a) and the *Safe Drinking Water Act* Amendments of 1996 (United States Government, 1996b), the US EPA began to implement a program to screen pesticide chemicals and environmental contaminants for their potential to affect the endocrine systems of humans and wildlife based on recommendations from the U.S. Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) (US EPA, 1998a, 2010d).

The resultant US EPA Endocrine Disruptor Screening Program (EDSP) is an example of a first-generation IATA approach approved for use in a regulatory context. It uses a tiered approach to screen pesticides, chemicals, and environmental contaminants for effects that are mediated by interactions with estrogen, androgen, or thyroid hormone signalling pathways. The components of the battery were selected based

on the capacity of the assays to detect estrogen- and androgen-related effects by various MoAs, including receptor binding, gene activation, hormone synthesis, and hypothalamic-pituitary-gonadal feedback.[119] Limitations in the capacity of *in vitro* systems to reflect aspects of xenobiotic metabolism and physiological feedback systems led to the inclusion of both *in vitro* and *in vivo* assays.

The EDSP adopts a two-tiered approach. In tier 1, chemicals are screened for their potential to interact with the endocrine system. In tier 2, the specific effect elicited by each endocrine-disrupting chemical is evaluated, and the dose at which the disruption occurs is identified. The tests used in the US EPA EDSP include *in vivo*, *in vitro*, and *in silico* assays that are complementary and run in parallel in order to screen substances for their ability to interact with the estrogen, androgen, and thyroid hormone systems. The results of the tier 1 battery are evaluated using a weight-of-evidence approach and will determine which (if any) of the tier 2 tests are required. Tier 2 tests confirm and characterize the effects identified in tier 1 and establish a quantitative dose-response relationship.

The suite of tier 1 assays has been developed, validated, and published (US EPA, 2009a). It includes five *in vitro* tests (estrogen- and androgen-receptor binding, estrogen transcriptional activation, steroidogenesis, and aromatase activity) and six *in vivo* tests (rat uterotrophic, Herschberger, and male and female pubertal together with amphibian metamorphosis and a short-term reproduction study in fish) (Table 4.2). Tier 2 assays are still undergoing validation.[120]

The EDSP focuses on a small group of chemicals for initial screening through the tier 1 battery of tests. A draft list of 73 pesticide active ingredients and HPV chemicals to be evaluated by the tier 1 screening was published in 2007 (US EPA, 2007b) and updated in 2009 for a final tally of 67 (US EPA, 2009m). This list was developed after evaluating the exposure potential and exposure pathway for each chemical. The test orders were issued in October 2009 (US EPA, 2009a), and a final response is due within two years. In an effort to minimize duplicate testing, the US EPA permits registrants to submit existing data in their initial response (reviewed in Bergeson, 2009).[121] A second list of 134 chemicals and substances for which the US EPA intends to issue test orders was published in November 2010 (US EPA, 2010e).[122]

119 The tests approved for use in the current US EPA EDSP battery do not provide MoA data for thyroid disruption.

120 Regular updates on the status of validation of tier 2 tests may be found at: http://www.epa.gov/scipoly/oscpendo/pubs/assayvalidation/status.htm

121 Defined by the US EPA as "testing the same chemical using the same test."

122 The list may be viewed at: http://www.epa.gov/endo/pubs/draftlist2.pdf

Table 4.2
Complementary modes of action evaluated by the EDSP tier 1 assays

Screening Assays	Modes of Action								TG Reference
	Receptor Binding				Steroidogenesis				
	E	Anti-E	A	Anti-A	E	A	HPG Axis	HPT Axis	
in vitro									
ER binding	X	X							(US EPA, 2009e)
ER-a transcriptional activation	X								(US EPA, 2009f)
AR binding			X	X					(US EPA, 2009c)
Steroidogenesis					X	X			(US EPA, 2009k)
Aromatase					X				(US EPA, 2009d)
in vivo									
Uterotrophic (female rat)	X								(US EPA, 2009l)
Hershberger (male rat)			X	X		X			(US EPA, 2009h)
Pubertal male (rat)			X	X		X	X	X	(US EPA, 2009j)
Pubertal female (rat)	X	X[4]			X		X	X	(US EPA, 2009i)
Amphibian metamorphosis (frog)								X	(US EPA, 2009b)
Fish short-term reproduction	X	X[4]	X	X	X	X	X		(US EPA, 2009g)

(US EPA, 2010n)

The screening assays listed in this table encompass certain key events within a mode of action (e.g., receptor binding) as well as certain pathways (e.g., steroidogenesis) through which a chemical can interact with the E (estrogen), A (androgen), HPG (hypothalamic-pituitary-gonadal) axis or HPT (hypothalamic-pituitary-thyroid) axis hormonal systems.

At the time of inception, high-throughput screening (HTS) and *in silico* approaches to categorization were considered insufficiently developed to use in a regulatory context for data-rich chemicals such as pesticides (reviewed in Reif *et al.*, 2010; US EPA, 2009r). As a result, the 67 chemicals that were identified for tier 1 screening were screened solely on the basis of exposure estimates; however, there have been significant advances in both computational and molecular technologies for discerning AOPs in the years since US EPA began work on developing and implementing the EDSP. Endocrine screening is viewed as a prototype for the use of molecular-based screening and computational methods. The US EPA

"anticipates that it may modify its chemical selection approach for subsequent screening lists" to include ToxCast™ assays and (Q)SAR analyses (US EPA, 2009r, 2010d). Indeed, the 2012 President's Budget for the US EPA explicitly includes provisions to modernize the assay composition and to use HTS to help prioritize the selection of chemicals to enter the screening process (US EPA, 2011). To this end, (Q)SAR models and proof-of-concept tools such as ToxPi™ (Box 4.5) are invaluable exercises that show the utility of predictive models and HTS assays.[123]

Box 4.5
CASE STUDY: ToxPi™

ToxCast™ is developing a large battery of HTS assays in order to identify activity signatures that can be used to predict the potential toxicity of environmental chemicals and prioritize them for further testing. Subsequent testing may then include *in vivo* or *in vitro* assays (Dix *et al*., 2007).

Within the ToxCast™ program, the Toxicological Priority Index (ToxPi™) was developed to facilitate the prioritization of chemicals. It is a graphical framework that can integrate data from diverse sources in a scientifically robust, transparent, and easy-to-interpret fashion. As a complement to the EDSP, 90 assays, two chemical properties, and 27 metabolic pathways were identified as having putative endocrine relevance and were selected for use in a proof-of-concept study to screen 309 chemicals (Reif *et al*., 2010).

In vitro targets were mapped to human genes in order to connect assays to endocrine-relevant pathways selected from a variety of database sources (reviewed in R. S. Judson, Houck *et al*., 2010; Reif *et al*., 2010). If a chemical was active in at least five assays that mapped to a single pathway, it was flagged and assigned a "pathway perturbation score." These scores were used to calculate a ToxPi™, a dimensionless index score, that represents a formalized and rational integration of all of the data collected. The ToxPi™ can be depicted graphically (Figure 4.5) with each data type (or domain) represented by a different colour group and each individual component (or aggregate of components) represented by a separate slice (Reif *et al*., 2010).[124]

continued on next page

123 It is important to note that any advance high-throughput molecular screening and computational profiling methods would need to be subjected to an evaluation and peer review of the underlying assumptions, relevance, reliability, sensitivity, and specificity prior to regulatory acceptance.

124 A domain refers to the field of knowledge (e.g., chemical properties, *in vitro* assays, or cellular pathways). A component (or pie slice) refers to the specific assays or properties that were measured.

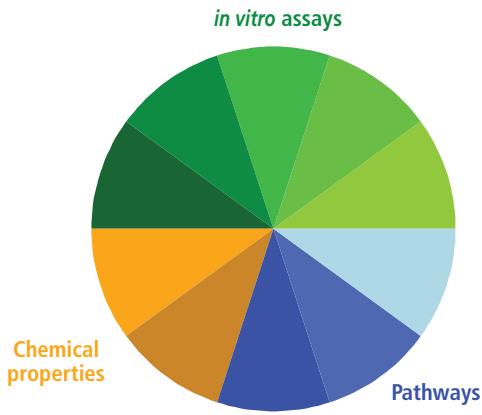
Box 4.5 *(continued)*

The ToxPi™ scores correlated well when compared to existing data on reference chemicals (i.e., those which already had a considerable body of toxicity data) that span the range of endocrine disruption potential (Figure 4.6).

This proof-of-concept study demonstrated the utility of an approach that permits integrating data from diverse domains into a framework to help prioritize chemicals for subsequent testing. Furthermore, the ToxPi™ visual profile permitted a transparent summary of the underlying rationale behind a prioritization decision.

In its current form, ToxPi™ is a research tool intended to help interested parties visualize the data. As such, its only assumption is that the underlying data is reliable and relevant. Key pathways relevant to endocrine disruption are well-known, which makes it an ideal proof-of-concept example of how various data domains can be integrated. As more data are generated, it can be used to expand the suite of assays used to inform this kind of integrated approach, thus enhancing its statistical and biological robustness.

This kind of approach makes it readily able to incorporate other domains of data, including (Q)SAR predictions, exposure information, and data from *in vivo* studies. This flexibility will be important as the body of chemical toxicity information grows following the legislative mandates of programs such as REACH.

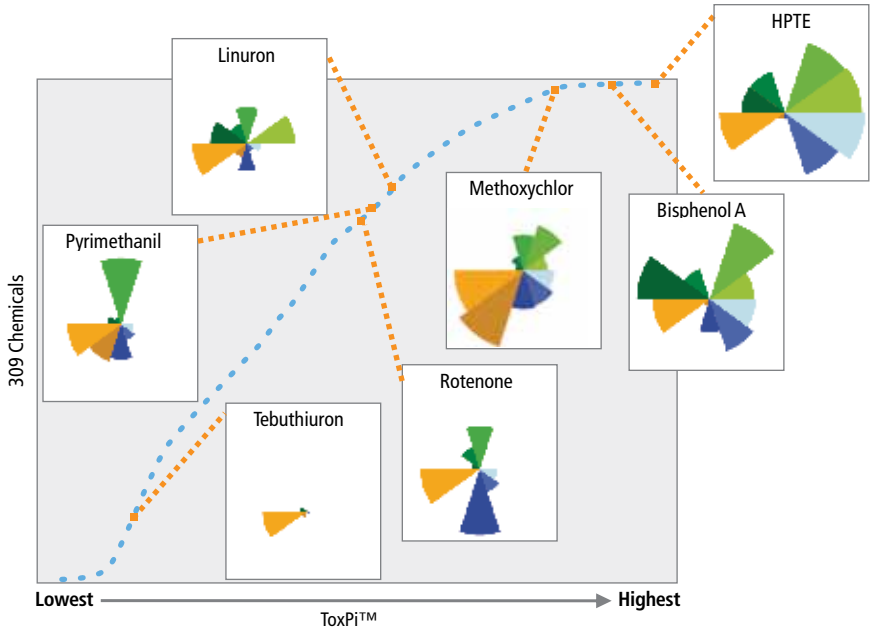


(Reproduced with permission from Gangwal, 2010)

Figure 4.5

A ToxPi™ can be depicted graphically

Domains are basic data types that are represented by a specific colour family. Green indicates *in vitro* assays; orange indicates chemical properties; blue indicates pathways.



(Reproduced with permission from Reif *et al.*, 2010)

Figure 4.6

ToxPi™ as a screening tool

The dotted blue line maps the 309 chemicals sorted by ToxPi™. The seven reference compounds presented in this figure demonstrate the potential utility of ToxPi™ as a screening tool.

As an example of a first-generation IATA, the EDSP is a pioneering program that many regulatory agencies around the world will watch carefully. The lessons learned in its development and implementation will surely be invaluable to other initiatives (for example, see the OECD Conceptual Framework for testing and assessment of potential endocrine disruptors, Box 4.6).

Box 4.6
The OECD Conceptual Framework for Testing and Assessment of Potential Endocrine Disruptors

The international community, through the OECD Endocrine Disruptor Testing and Assessment Advisory Group, recognized the importance of relatively inexpensive and quick screens as well as tests to evaluate chemicals with the potential to cause

continued on next page

Box 4.6 *(continued)*

endocrine disruption. The OECD developed a conceptual framework to provide a tiered and resource-efficient approach for the testing and assessment of endocrine-disrupting chemicals (OECD, 2002a).

Level 1: Sorting and prioritization based on existing information	• Physical and chemical properties • Human and environmental exposure • Hazard
Level 2: *in vitro* assays providing mechanistic data	• ER, AR, TR receptor binding activity • Transcriptional activation • Aromatase and steroidogenesis • (Q)SARs • HTS Prescreens • Thyroid function • Fish hepatocyte VTG assays • Others as appropriate
Level 3: *in vivo* assays for single endocrine mechanisms and effects	• Uterotrophic and Hershberger assays • Non-receptor mediated hormone function • Fish VTG assay • Other assays (e.g. thyroid)
Level 4: *in vivo* assays for multiple endocrine mechanisms and effects	• Enhanced OECD 407 • Male and female pubertal assays • Adult intact male assays • Fish gonadal histopathology and frog metamorphosis assays
Level 5: *in vivo* assays for effects from endocrine and other mechanisms	• Full and partial life cycle assays to study developmental and reproductive effects • Enhanced 1-G and 2-G assays • Reproductive screening test • Combined 28-day reproduction screening test

(Adapted from OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals, OECD 2002, www.oecd.org/env/testguidelines/framework)

OECD Conceptual Framework for the testing and assessment of endocrine-disrupting chemicals

The framework is structured into several tiers or levels. The initial level is a sorting and prioritization step; thereafter, each screening and testing tier progresses to a different level of biological complexity (e.g., *in vitro* versus *in vivo* testing) for both toxicological and ecotoxicological areas. A chemical may enter or exit the framework at different levels depending on the nature of existing information and the needs of the regulatory decision.

During the Deepwater Horizon oil spill, over one million gallons of oil dispersants were released into the Gulf of Mexico between 20 April and 15 July 2010. Although some toxicity data were available on the dispersants, the tests had been conducted by different laboratories and on a different type of oil to that released into the Gulf of Mexico. In order to facilitate a better comparative analysis and to ensure that all available data were used to inform a rapid decision on the best dispersants to use in that situation, the US EPA conducted a series of toxicity tests in a single laboratory using the oil from the Gulf of Mexico (US EPA, 2010k). These toxicity tests were conducted in two phases. The first phase used a rapid battery of *in vitro* assays and acute toxicity tests in order to evaluate the toxicity of eight dispersants. The second phase evaluated acute toxicity of the oil alone and the chemically dispersed oil in two sensitive Gulf of Mexico aquatic species.

In phase 1, a diverse battery of tests developed through the ToxCast™ and Tox21™ programs was used in order to probe the dispersants for their capacity to interact with targets in a number of toxicologically relevant metabolic pathways (R. S. Judson, Martin *et al.*, 2010). Specifically, the results of these tests were intended to indicate (US EPA, 2010f):

- potential endocrine-disrupting activity;
- cytotoxicity to mammalian cells; and
- acute toxicity to shrimp and fish.

The eight test compounds were evaluated alongside a total of 23 reference compounds that served as experimental controls (R. S. Judson, Martin *et al.*, 2010). These reference chemicals had previously been approved for use as controls in endocrine disruptor screening assays by other organizations (ICCVAM, 2006a; US EPA, 2009o). The results of the HTS assays suggested that none of the eight dispersants tested exhibited biologically significant endocrine disruption activity, and cytotoxicity was only observed at concentrations above 10 ppm (R. S. Judson, Martin *et al.*, 2010). These *in vitro* data were supported by the ecotoxicology results, which also showed generally low dispersant toxicity (Hemmer *et al.*, 2010a).

The results of the phase 2 assays conducted in two sensitive Gulf of Mexico aquatic species suggested that all eight dispersants exhibit similar toxicity profiles and that the dispersant that was used in the disaster is no more or less toxic than the others (Hemmer *et al.*, 2010b). What is particularly noteworthy is that the results of these phase 2 toxicity tests showed that the toxicity of dispersants alone was much lower than that of the combined oil-dispersant mix; indeed, the acute toxicity of the dispersant-oil mixture was comparable to that of the oil alone.

The use of HTS in the evaluation of dispersants after the Deepwater Horizon oil spill disaster provides a good example of the potential value of this approach. The assays were able to rapidly provide bioactivity data on complex mixtures of chemicals, showing, for the first time, their efficacy and capability in an emergency. This is particularly exciting given that one of the main challenges in the toxicological evaluation of chemicals is that of complex mixtures. Nonetheless, the deployment of these tools represents a policy decision, rather than a scientific one, because no viable alternative approach existed. The data were not used in a formal quantitative risk assessment to approve or cancel a substance; rather, they represented a hazard comparison of biological activity among dispersants.

The Use of Integrated Approaches in Ecotoxicology:

Ecotoxicology is concerned with the toxicological effects of chemical exposure on all species and levels of biological complexity (Box 4.7). Although environmental risk assessment (ERA) and human health risk assessment (HHRA) share many of the same basic properties, ERA requires understanding the physical/chemical properties of compounds, their environmental fate, and their adverse effects on humans, aquatic and avian species, and other wildlife. Because human anatomy, physiology, and biochemical processes are quite different from those of wildlife and plants, the underlying hazard identification and exposure assessment steps are quite distinct. In order to meet the mandates of the *Clean Water Act* (CWA) (United States Government, 1972a) and the *Federal Insecticide, Fungicide, and Rodenticide Act* (FIFRA) (United States Government, 1972b), the US EPA has been developing a Common Effects Assessment Methodology (US EPA, 2009p). The goal is to develop a common framework that is focused on data-limited situations. This framework would allow regulators to take advantage of all available data in a consistent and transparent fashion. Furthermore, this framework would be used to complement current approaches to ERA by assessing uncertainties in interspecies sensitivity in aquatic ecosystems (US EPA, 2009p). Tools that might be used as part of this initiative include (Q)SAR, read-across, chemical categorization, MoA, and interspecies correlation models (US EPA, 2010a). Indeed, computational approaches have long been accepted in ecotoxicology, where (Q)SAR approaches and *in chemico* testing are used to estimate physicochemical properties and toxicological endpoints associated with aquatic and acute effects resulting from exposure to pesticides and pesticide formulants as well as other industrial chemicals (Bradbury, 1995).

4.1.4 Current International Uses of the Threshold of Toxicological Concern in a Regulatory Context

There are numerous examples of the use of the threshold of toxicological concern (TTC) approach to inform regulatory decision-making across a wide range of

Box 4.7
An Aside on Environmental Risk Assessment

Environmental Risk Assessment (ERA) is designed to protect populations. It is assumed that by protecting most of the species the functioning of an ecosystem is also protected. There is general agreement that there are a number of unacceptable toxic effects on populations, including unacceptable reductions in survival, growth, and reproduction. As a result, environmental toxicity testing traditionally focuses on endpoints that are associated with these three effects.

Laboratory testing for environmental toxicology is typically conducted using indicator organisms from different taxonomic groups. Toxicity is measured by studying lethal (i.e., death) and sublethal effects (e.g., reduced growth, and organ toxicity) in order to calculate a No Observed Effect Concentration (NOEC) (PMRA, 2000). ERA also deals with the sustainability of ecosystems, which necessitates temporal scales that can be quite different to those assumed for human health risk assessment. Human-based time frames are long compared to the life spans of many species. In environmental toxicology, the objective of acute testing is to determine the concentration of a particular toxicant that elicits a specific response or measured endpoint (e.g., death) in a relatively short period of time (e.g., a week or less). In contrast, chronic toxicity studies look at effects over the life cycle of the test organism. Organisms with short life cycles (e.g., algae, protozoa) are often exposed over their life span and the endpoints measured are sublethal (e.g., growth or reproductive output).

Environmental exposure is estimated by modelling expected environmental concentrations (EEC) using information on the physicochemical properties and transformation rates of the substance.[125] Estimating environmental exposure can be particularly challenging because of the existence of numerous confounding factors. These factors might include exposure duration (particularly in relation to a particular species and its generation time), niche partitioning, food consumption patterns, metamorphic lifecycles, and life stage exposure.

ERA combines the results of the environmental toxicology studies with the environmental exposure data in order to quantify environmental risk. This is often done by comparing the ratio of the NOEC to the EEC; a larger ratio indicates a bigger margin of safety and a lower environmental risk (PMRA, 2000).

125 In the European Union, the EEC is referred to as the Predicted Environmental Concentration (PEC).

chemical classes and regulatory jurisdictions[126] particularly for those substances present at low levels in foods, cosmetic ingredients, household products, pest control products, and pharmaceuticals (Figure 4.7 and Box 4.8) (Blackburn *et al.*, 2005;

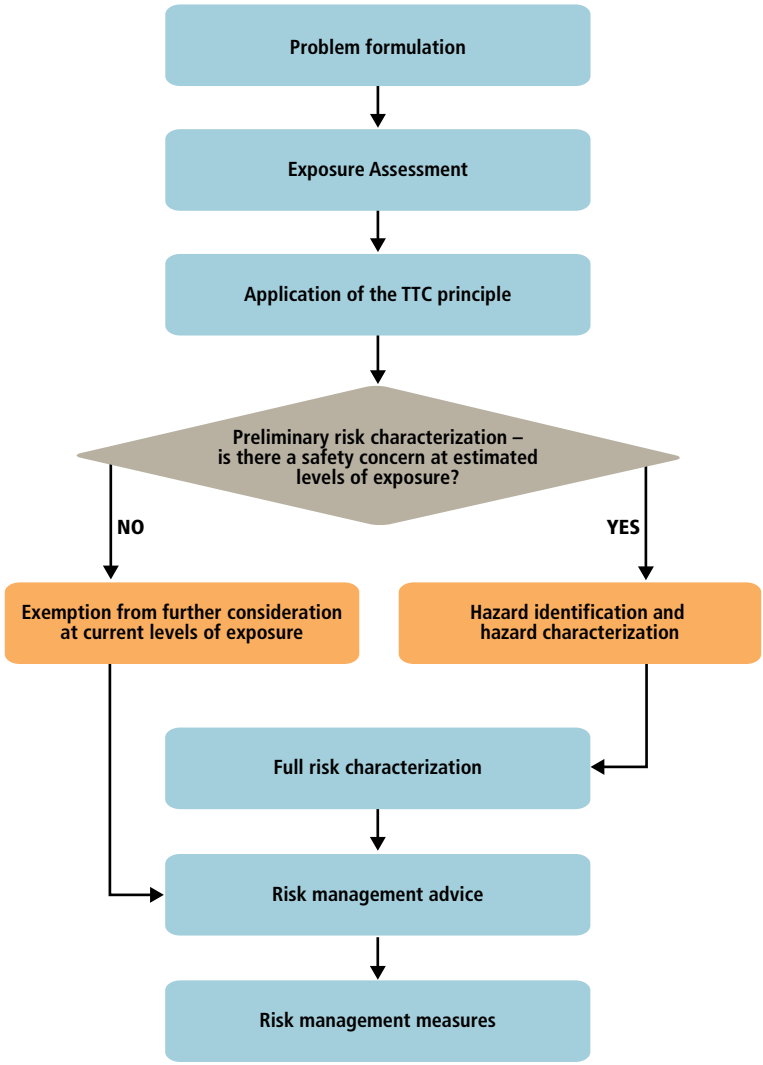


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Figure 4.7
Schematic representation of the application of the TTC in a risk assessment process

126 See Munro *et al.*, 2008 for a more comprehensive review.

Felter *et al.*, 2009; Kroes *et al.*, 2007; Munro, 1996; Munro *et al.*, 1996; Munro *et al.*, 2008). Indeed, one of the earliest examples of the regulatory adoption of a TTC-like concept may be found in Canada, where a default Maximum Residue Limit (MRL) for food residues of 0.1 ppm was adopted under the mandate of the *Food and Drugs Act* (Government of Canada, 1985c).[127] This concentration of 0.1 ppm was assumed to be a safe level of exposure because it represented:

- the practical analytical detection limits at the time of adoption, and
- the level below which exposures were considered to be toxicologically irrelevant.

Box 4.8
The Threshold of Toxicological Concern for Low-Dose Chemicals

The threshold of toxicological concern (TTC) assumes that a safe level of exposure can be identified for individual chemicals with known toxicological profiles. As such, it is an approach that sets a *de minimis* value below which exposure is considered unlikely to be of concern. This assumption is based on the structural characterization of the chemical in question and existing toxicity data for other (related) substances in an identified database. The TTC has received particular attention in the area of food and food-related products. For example, it was predicted that establishing an accepted TTC for food-contact articles would preclude extensive toxicity evaluations, thereby freeing up resources for the testing of substances with the highest potential risk to human health (Kroes *et al.*, 2000). Yet questions remained about whether low-dose effects could exist that would not be identified using the TTC approach. In their work, Kroes *et al.* (2000) evaluated a number of toxicity endpoints to see whether this was indeed the case.

For each endpoint, Kroes *et al.* (2004) developed a database of specific NOEL data from oral toxicity studies on specific substances. The subsequent analyses, conducted using conservative "worst-case" assumptions, concluded that the TTC approach could be used to define a safety threshold for low-dose food chemicals that lacked primary toxicity data but which had adequate exposure data (Kroes *et al.*, 2004). Chemical substances consumed at levels below this threshold were considered to pose no appreciable risk.

A decision tree was subsequently developed to help consistently apply TTC principles to low-dose chemicals. This decision tree incorporates different TTCs based on structural

continued on next page

127 In 2006, Health Canada proposed the revocation of the default 0.1 ppm limit (PMRA, 2006a); however, a precise timeframe for this transition has yet to be defined (PMRA, 2009a).

***Box 4.8** (continued)*

characteristics of the chemicals in question (Kroes *et al*., 2005; Kroes *et al*., 2004). The TTC decision tree outputs are either that:

- the anticipated exposure is not predicted to represent a safety concern; or
- a risk assessment is not appropriate without further toxicity data.

These outputs are intended to accelerate the evaluation of low-dose chemicals and provide risk assessors and regulators with practical tools to prioritize chemicals for additional testing (Kroes *et al*., 2004).[128]

The US FDA adopted a "threshold of regulation" of 0.5 ppb for indirect food additives that were neither shown to be carcinogenic nor possessed structural alerts that indicated potential genotoxic carcinogenicity (US FDA, 1995). In addition, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) adopted a TTC approach to evaluate flavouring substances (JECFA, 1997) using TTC values for three structural classes of chemicals using existing toxicity data (Munro *et al*., 1996). Since then, over 1,600 chemicals have been evaluated using this approach (reviewed in Munro *et al*., 2008).

More recently, the European Food Safety Authority (EFSA) formed a scientific working group to investigate broadening the TTC concept to include metabolites, degradation products, and reaction products of active substances of plant protection products (Brown *et al*., 2009; CTG, 2010).[129] Also, the International Life Sciences Institute (ILSI) Research Foundation continues to explore framework and decision trees that incorporate a TTC-based approach as a risk assessment tool for antimicrobial pesticide active ingredients and to address higher-tier toxicology data requirements for these substances.[130]

There are many efforts to investigate the TTC concept, especially with respect to refining the definition of the chemical space represented by existing TTC values and refining the existing Cramer classes, which are quite crude. Furthermore, there is a great deal of activity around establishing toxicity data repositories combined with chemical structure information. The goal is to facilitate screening

128 IHCP: http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree
129 EFSA: http://www.efsa.europa.eu/
130 ILSI: http://www.ilsi.org/

or prediction of potential chemical effects with little or no data (e.g., ToxRefDB). The TTC database could be expanded with toxicity data for important endpoints of concern across a range of chemical classes of interest. TTC values are based on oral exposure, and thus dermal or inhalation exposures would need to be appropriately addressed.[131]

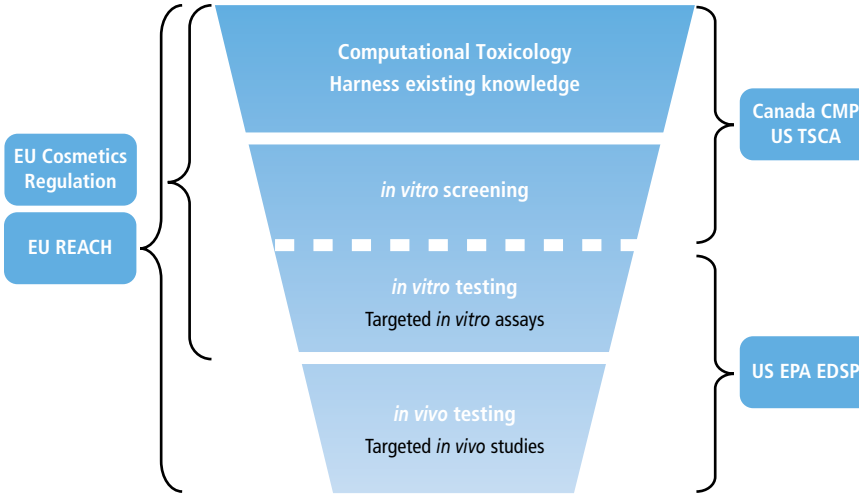
Assuming that the chemical space is well represented, the Panel believes that the TTC concept is a scientifically viable approach for putting low-level exposures of data-poor substances in the context of potential risk and to facilitate testing prioritization. There is no *a priori* reason why the TTC concept could not be used to address data-limited situations for pesticide formulants. This approach provides another screening tool that could be applied to data-poor substances such as the so-called "inert" ingredients or metabolites/degradates of active ingredients to determine the need for chemical-specific animal toxicity data. Exposure to residues shown to be present below the TTC values could then be exempted from further testing or regulation. Combined with appropriate structural information, these TTC values could form the basis of a hypothesis-driven testing approach for data-poor chemicals in a tiered risk-assessment scheme.

4.1.5 Summary of the Status of Regulatory Implementation

There are a number of examples of the use of components of IATA in a regulatory context for industrial chemicals and personal care products; however, there is no single example of a comprehensive hierarchical deployment of IATA in a regulatory context. Of the examples illustrated in the preceding section, only REACH provides a flexible framework that encourages or requires the use of alternative testing approaches that are consistent with the Panel's interpretation of an IATA approach (Figure 4.8).

The purpose of regulatory toxicity testing is to protect human health and the environment. Governments have an ethical obligation to ensure that regulatory decisions are made using the best available scientific information. As a result, the two primary drivers of regulatory reform in toxicity testing are legislative reform and ethical obligation. This combination will likely be a powerful driver of innovation for regulatory toxicology; however, the current approach to testing is entrenched because it provides familiarity and security for industry and regulators. This is especially true for heavily regulated, internationally harmonized, data-rich chemicals such as pesticide active ingredients where changes in the data requirements would require international coordination and agreement.

131 It is important to note that the current TTC concept does not allow for assessment of local effects such as respiratory or skin sensitization.



(Adapted and reproduced with permission from Taylor & Francis Group and Dellarco, Henry *et al.*, 2010)*

Figure 4.8
Only REACH mandates the use of a full IATA approach

*Meeting the Common Needs of a More Effective and Efficient Testing and Assessment Paradigm for Chemical Risk Management, Vicki Dellarco, Tala Henry, Phil Sayre *et al.*, *Journal of Toxicology and Environmental Health, Part B: Critical Reviews*, 2010, Taylor & Francis, reprinted by permission of the publisher (Taylor & Francis Group, http://www.informaworld.com).

The widespread deployment of new testing tools will therefore necessitate the negotiation of a number of hurdles. Although the specific nature of these hurdles will depend on the current testing requirements of the chemicals in question (data-rich versus data-poor), these hurdles could include practical, legal, communication, and scientific considerations.

The deficit in toxicity data for the majority of commercial industrial chemicals represents a significant limitation to the existing approach, and one that the introduction of legislation is starting to address. Legislative mandates that increase the data requirements for chemicals using the existing paradigm will increase both the economic burden and the number of animals needed. Initiatives such as REACH do permit and encourage the use of alternative tests; however, the validation of these tests has limited the rate of their deployment in the regulatory setting. As illustrated by the example of the EU Cosmetics Regulation, the assignment of a deadline that mandates the use of alternative tests is impractical if the deadline is not realistic.

The challenge of test development and regulatory acceptance is an issue that has received considerable attention over many years. The validation process itself can take many years; however, the nature of the validation process precludes the expeditious revision of test protocols that better reflect the state of the art in scientific understanding. The Panel believes that the pace of scientific progress in the so-called omics era, coupled with the challenge of increased data requirements for chemicals, necessitates a fundamental shift in the way the validation process is approached.

4.2 SCIENTIFIC VALIDATION AND REGULATORY ACCEPTANCE OF IATA TESTS

Before alternative testing strategies can be used in a regulatory context, they must demonstrate both their scientific reliability and predictivity. Scientific validation ensures that alternative tests will yield credible results and provide suitable data for fair and proper regulation. Prior to being used in practice, however, the various validated protocols must also meet the approval of regulating bodies throughout the world. Validation of an alternative testing method is a prerequisite to — but not a guarantee of — regulatory acceptance and implementation.

The current approach to validation has a number of limitations that impede the development and approval process for *in vitro* and alternative testing approaches. The purpose of this section is to introduce those limitations and suggest pragmatic approaches to address them.

4.2.1 The Current Approach to Validation

The principles and criteria for the validation of alternative test methods were developed by three principal validation authorities: the Organisation for Economic Co-operation and Development (OECD) (Box 4.9), the European Centre for the Validation of Alternative Methods[132] (ECVAM), and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM).[133;134] All three of these bodies cooperate closely and conduct evaluations according to the same fundamental principles. Proposed test methods are assessed based on their purpose, relevance, mechanistic basis, and performance when compared to existing regulatory tests, reproducibility within and among laboratories, and adherence to good laboratory practices (Bruner *et al.*, 1998; Worth & Balls, 2004).

132 ECVAM: http://ecvam.jrc.ec.europa.eu/

133 ICCVAM: http://iccvam.niehs.nih.gov/

134 Canada does not have its own validation organization but is a part of the International Cooperation on Alternative Test Methods (ICATM) along with the U.S., EU, and Japan (ICATM, 2009).

Box 4.9
CASE STUDY: The Role of the OECD

Although it is not a regulatory body, the OECD is the pre-eminent international source of chemical testing guidelines used by government (including the Pest Management Regulatory Agency (PMRA) at Health Canada), industry, and other laboratories. It provides broad guidance documents for validation procedures and specific test guidelines governing the proper application of a validated method. Both ICCVAM and ECVAM participate in the development of OECD standards and guidelines and cooperate to further the goal of greater international harmonization (OECD, 2005).

At the OECD, chemicals management and the validation/acceptance of testing strategies fall under the mandate of the Environment Directorate. The chemicals program is a collaborative effort, with input from 30 member countries, to oversee and manage chemical- and pesticide-related activities. In 2005, the Environment Directorate published its *Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment*, which sought to outline the promotion of the "harmonization of international regulatory acceptance of adequately validated test methods" (OECD, 2005). The principles and criteria described in the OECD document are based on those established by ECVAM and ICCVAM, as well as other acknowledged validation bodies; the criteria for regulatory acceptance are summarized in the table below.

OECD principles for the regulatory acceptance of alternative test methods

Criterion	Description
Peer review	The submitted test method and supporting data must have been subject to a transparent and independent peer review.
Endpoint-specific	The data generated must adequately measure or predict the endpoint of interest.
Usefulness of data	Data must be useful for hazard/risk assessment purposes. New test methods may fill a data gap. Substitute methods must be at least as useful as (and preferably better than) the methods they replace.
Coverage of chemical space	Supporting data must cover the spectrum of chemicals that might be tested by the method in a regulatory context.
Robustness	The test method must be sufficiently robust and demonstrate inter-lab consistency.
Time and cost-effectiveness	The test must be time and cost effective and likely to be used in a regulatory context.
Justification	The need for the alternative test must be justified.

continued on next page

Box 4.9 *(continued)*

In 2007, the OECD published a monograph outlining six principles for the validation of (Q)SAR models (OECD, 2007b). The approach described in that monograph placed the onus on the test proponent to determine its relevance and adequacy according to their defined needs. Although a detailed discussion of this approach is outside of the scope of this assessment, the approach may be relevant to the validation of *in vitro* assays.

Table 4.3

Four main scientific components currently considered in the regulatory approval process for an alternative test method

Criterion	Description
Relevance	The assay data are useful to the evaluation of the endpoint of interest.
Protocol	The assay has a detailed protocol that includes material and equipment requirements, measurement procedures, controls, and test limitations.
Reliability	Assay results are reproducible over time and space; test protocols include defined positive and negative controls that can be used to evaluate the experimental set up and performance.
Verifiability	Data, methods, and protocols are published in a physical medium that enforces independent, peer-review processes.

Although the coordinated efforts of the OECD, ICCVAM, and ECVAM speak to the success of international harmonization initiatives in regulatory toxicity testing, the validation process outlined above is very slow. There is a need for a concerted initiative to reconsider what the scientific validation of an alternative test really means and to develop more efficient processes to ensure that the validation process itself is not a barrier to scientific progress and regulatory change.

The Panel believes that validating an alternative test method against the data produced by an *in vivo* assay (that itself may not have been subject to a rigorous and stepwise validation process) speaks to a fundamental flaw in the approach to scientific validation. It is time to move away from thinking about alternative toxicity assessment approaches and their validation in terms of a one-for-one replacement of an existing animal study and towards a new approach that is anchored in an understanding of the underlying biology.

4.2.2 Moving Away from One-for-One Replacement and Towards Performance-Based Standards

In vitro toxicity tests that are targeted to specific physiological responses preclude validation by a one-for-one approach. An array of assays would be designed to target a specific pathway, and each assay in that suite should produce data that would inform the next level of the decision-making process. This, however, conflicts with the existing approaches to validation espoused by the OECD and ECVAM.

The OECD definition of an endpoint as a "test protocol endpoint" infers that any validated test (or battery of tests) must be developed and validated as a one-for-one replacement of an existing protocol. The current principles for validation suggest that the acceptance of a test battery should be predicated on its overall performance for the intended purpose (OECD, 2005). In 2009, the ECVAM Scientific Advisory Committee (ESAC) released a statement that read: "The replacement of traditional animal-based test methods by alternative ones should ideally be obtained by one-to-one replacements: to keep the testing regime simple and economical one single alternative method should, wherever feasible, be sufficient to generate data of equal or better quality than the traditional test" (ESAC, 2009). This statement highlights an important issue. Many alternative tests, especially those that are based on mode of action, will not lend themselves well to validation in this manner. For example, in the case of a replacement test to assess ocular irritancy (an *in vivo* endpoint that was due to be phased out under the EU Cosmetics Regulation by March 2009), no validated *in vitro* approach has been developed that can accurately replicate the *in vivo* endpoint. In recognition of this, ESAC has acknowledged that, for the foreseeable future at least, a battery of *in vitro* tests will likely be needed (ESAC, 2009).

The Panel anticipates that, as our mechanistic understanding of the underlying physiology of a toxicological response increases, assays designed to evaluate a number of points along the toxicity pathway will be developed. These assays will likely be *in vitro* and will certainly not lend themselves well to validation against endpoint-driven criteria. Indeed, the eye irritancy test is an excellent example of how the development of an alternative test against a specific endpoint (eye irritation) is less likely to be successful than the development of alternative tests for the individual processes involved (Box 4.10).

Box 4.10
CASE STUDY: Regulatory Acceptance of Alternative Tests for Eye Irritation

Developing a validated *in vitro* replacement for the Draize eye irritation test has proven to be a challenge, although some tests have been used in industry for specific purposes (reviewed in Scott *et al*., 2010). This is especially prescient given the legislative requirements to eliminate *in vivo* testing of cosmetics under the EU Cosmetics Directive.

ECVAM held a meeting in 2005 to review limitations in and opportunities for developing *in vitro* eye irritation tests in order to devise an integrated strategy that could be used in a regulatory environment. The applicability domain for the eye irritancy test was defined as the mechanism by which the substance of interest induced eye irritation. This was determined to be by one of four MoAs:

- cell membrane lysis;
- coagulation due to precipitation of macromolecules;
- saponification due to lipid breakdown by alkaline action; or
- actions on macromolecules.

Specific *in vitro* tests were nominated based on their appropriateness to address these MoAs. Using these mechanisms as a starting point, an Integrated Testing Strategy was proposed (Figure 4.9).

The availability of validated *in vitro* tests for eye irritation limit the utility of this approach at this time; however, two *in vitro* methods have been validated and accepted for use by U.S. and EU regulatory authorities (ESAC, 2007; ECB, 2006; ICCVAM, 2006b). Further work is underway to refine and validate additional tests that can be used within the framework depicted in Figure 4.9.

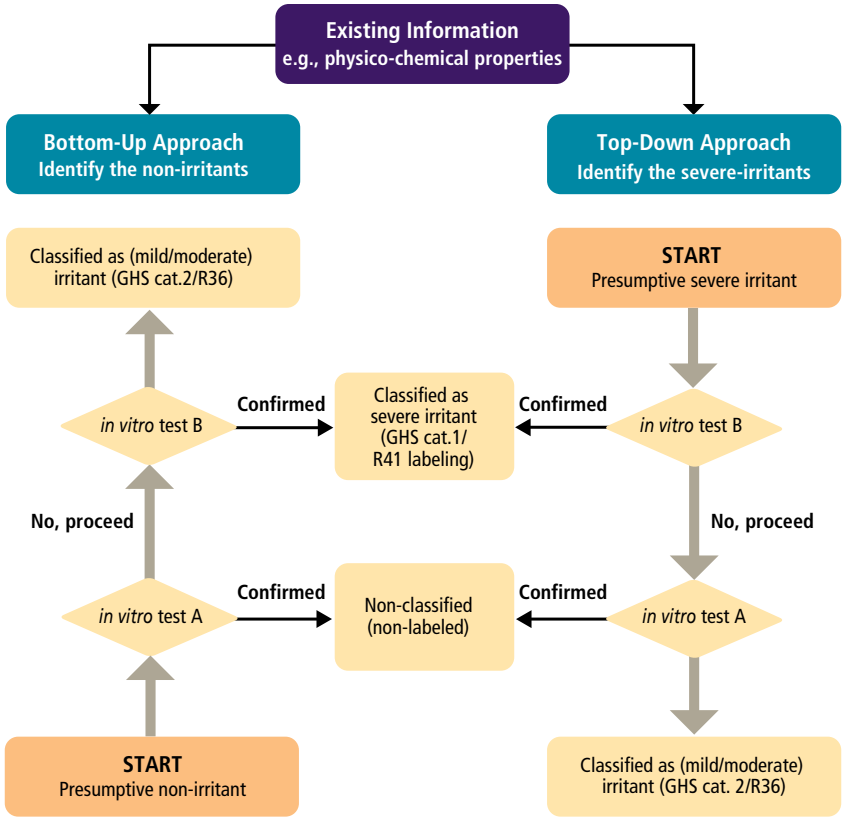


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Figure 4.9

An integrated approach to assess eye irritation

An initial assessment of the likely irritancy level of substances is made based on an assessment of existing data. If these data indicate that the substance is likely to be a severe irritant, a top-down approach is used; if it is predicted to have low irritancy, a bottom-up approach is more appropriate.

*Reproduced from: *Toxicology in Vitro*, 24/1, Laurie Scott, Chantra Eskes, Sebastian Hoffmann, Els Adriaens, Nathalie Alepée, Monica Bufo, Richard Clothier, Davide Facchini, Claudine Faller, Robert Guest, John Harbell, Thomas Hartung, Hennicke Kamp, Béatrice Le Varlet, *et al.*, A proposed eye irritation testing strategy to reduce and replace *in vivo* studies using Bottom-Up and Top-Down approaches, 1-9, Copyright (2010), with permission from Elsevier.

Alternative methods (either testing or non-testing) typically target specific cellular or physiological responses and, as such, preclude validation with *in vivo* data by a one-for-one approach. For example, an AOP allows for the use of a suite of models or assays (and subsequent databases) that target particular steps along a specific pathway. The scientific justification of an alternative method or data set

should therefore focus on comparing the test outcome to what is known about the underlying biology as described in the AOP. Each assay/data set in an array of information would inform the next tier of the IATA or be used as part of an overall integrated testing strategy. Not all key events in an AOP, all tiers in an IATA, or all aspects of integrated testing strategy have to be satisfied to make an assessment.

The Panel believes that the scientific validation of an alternative test method should be based on understanding the biological AOP or MoA. Alternative tests would therefore be validated against mechanistic endpoints. This is in contrast to the use of apical endpoints, which describe observable outcomes at the level of the test organism.[135] Mechanistic endpoints are those that can be measured in assays that are designed to evaluate a specific cellular or physiological response. The precise mechanism in question depends on the level of biological organization at which the phenomenon is observed. For *in vitro* assays, these mechanistic endpoints might include nuclear receptor binding, DNA damage, disruption of the cell cycle, and/or apoptosis.

Figure 3.8 described the integrated and iterative process for developing systems-level models of human biology. This kind of approach can be very useful for the performance-based validation of alternative tests; it is hypothesis-driven and makes use of extensive controls that demonstrate the experiment is working as expected by comparing the data that are generated against known physiological responses. The nature of the assay (i.e., genotoxicity, enzymatic assay, immunoassay, etc.) will determine the specific approach necessary to validate the scientific integrity of the testing method; however, the Panel believes that performance-based validation should be the defining principle by which all alternative test methods are judged.

4.3 ADDRESSING THE NEEDS OF REGULATORS AND THE REGULATORY PROCESS: THE NEED FOR FUNCTIONAL ENGAGEMENT

The current risk assessment processes are predicated on the types of data that have historically been generated by toxicity testing. The nature of the data generated by alternative testing methods may not be useful in the regulatory context. As a result, the Panel expects that the nature of an IATA strategy will vary depending on the type of chemicals in question and the nature of the decision-making process that the data are intended to inform.

135 The Panel recognizes that validation of alternative tests based on mechanistic endpoints presumes that there is a well-established relationship between the perturbation of the associated cellular pathway and an adverse health outcome.

4.3.1 Chemical Risk Assessment has Three Main Areas of Activity

As discussed in Chapter 2, information derived from hazard and exposure assessments may be used for priority-setting to identify those chemicals within a large group that should be considered for further work; in risk assessment to establish health-based values; and in hazard classification and product labelling (OECD, 2008c). Each of these activities has different data needs; therefore, the applicability and relevance of data from alternative testing tools differs depending on the regulatory activity in question.

Priority-Setting for Data-Poor Chemicals:

Data generated by integrated approaches — including non-animal methods such as (Q)SAR, category formation, read-across and *in vitro* assays — have historically played a role in priority-setting and screening to determine follow-up actions (or additional testing needs). They are expected to continue to evolve in a manner that would provide more accurate predictions. This will certainly be advanced by the evolution of some of the regulatory initiatives introduced earlier in this chapter. In addition, non-animal methods have also been used, and will continue to be considered, in the classification and labelling of acute hazards.

In the short term, initiatives such as the CMP and REACH are likely to significantly impact the adoption of IATA for the rapid screening and prioritization of chemicals. As a result, it is anticipated that data from alternative test methods (including high-throughput *in vitro* screens) might be used to fill data gaps and inform decisions about the need for higher-tiered *in vivo* data on specific chemicals (in order to confirm the predicted effects and to generate dose-response data for quantitative risk assessment). In the medium term, the Panel anticipates that data from HTS assays might be used in a manner comparable to how TTC uses data today. The use of HTS data in prioritization and screening, coupled with the development of prototype assay suites, would help develop confidence and familiarity with these approaches among the broader stakeholder community. In addition, the use of HTS assays in this manner would result in a large increase in the amount of primary data on otherwise data-poor chemicals. This would increase the amount of chemical-specific data, which in turn would facilitate efforts to understand the inherent toxicological properties of different chemicals.

Applicability to Quantitative Risk Assessment for Data-Rich Chemicals:

As discussed in Chapter 2, data-rich chemicals are already subject to an extensive battery of toxicity tests. Although adopting IATA strategies might refine and streamline the testing of these chemicals, the Panel does not anticipate a widespread deployment of IATA in the short term.

The data generated from HTS assays are well suited to the rapid screening of large numbers of chemicals; however, they cannot, at this time, replace the *in vivo* data currently used in the quantitative risk assessment process. This is partly due to inherent differences in the nature and objectives of screening versus testing (Box 4.11) and partly because the current risk assessment process for data-rich chemicals is based on the types of data that have historically been available. These data have been generated from *in vivo* tests designed to study apical endpoints. As described in Chapter 2, the current risk assessment process ultimately relies on calculating a numerical value, which means that the data generated from alternative tests do not necessarily meet the needs of risk assessors. As a result, although *in vitro*, *in silico*, and omics data may provide mechanistic insight and enhance the interpretation of traditional *in vivo* toxicological data, the use of alternative test methods to quantify risks and establish regulatory endpoints and exposures will remain a challenge.

Box 4.11
An Aside on Screening Versus Testing

Although both toxicity tests and toxicity screens make use of the same fundamental scientific understanding, they are not the same.

A screen is used to facilitate the rapid analysis of a large number of subjects (e.g., environmental chemicals) to identify any that may possess characteristics that warrant further investigation (e.g., endocrine disruption potential). Toxicity screens are highly sensitive; a sensitive screen with a negative result should indicate absence of toxicity.

A test is used to generate precise data on specific substances of concern (e.g., putative endocrine disruptors) in order to determine their underlying toxicological properties and their dose-response relationships (e.g., ER binding resulting in reproductive impairment), typically in an *in vivo* model. Toxicity tests are highly specific; a specific test with a positive test result should indicate an adverse outcome.

Over the next decade, *in vitro* assays will likely see regulatory use for those data-rich chemicals with reasonably well characterized MoAs, and for endpoints that cannot be easily assessed using *in vivo* tests. This is already apparent in their adoption for the rapid prioritization of HPV chemicals and the evaluation of endocrine disruption potential (reviewed in Combes *et al.*, 2006). These kinds

of proof-of-concept studies will therefore be invaluable in developing familiarity with the data, as well as confidence in their reliability, among regulators and decision-makers.

Other alternative approaches that may be used in a regulatory context in the long term are those that generate data that integrate into the existing requirements. For example, it is conceivable that functional genomics could radically alter our approach to defining threshold and non-threshold doses (Zarbl *et al.*, 2010). Functional genomics may permit the calculation of a new threshold level, the No Observed Transcriptional Effect Level (NOTEL) (Lobenhofer *et al.*, 2004) that, in the long term, could be a more sensitive indicator of biological relevance than NOEL or NOAEL (reviewed in Zarbl *et al.*, 2010). Furthermore, calculating NOTEL would permit incorporating these kinds of data into the current risk assessment framework if the link between the dose-response and adverse effects is understood.

In the short and medium term, the data generated from these screens can contribute tremendously to prioritizing chemicals for subsequent testing; however, the Panel believes that, in the long term, for these tests to replace the current battery of *in vivo* studies for data-rich chemicals, a fundamental change in the risk assessment process would be required. Although a discussion of how this change might evolve is outside of the scope of this report, the Panel expects it will be guided by methodological advancements and the evolution in understanding of toxicity pathways. As a result, a sustained and coordinated dialogue among scientists, regulators, and other key stakeholder groups will facilitate and inform the nature of this evolution.

4.3.2 The Importance of Functional Collaboration

While there is no silver bullet to transform the process for validation and regulatory acceptance, the Panel believes that it will necessitate early, sustained, and genuine dialogue between scientists and regulators. Assays should be developed with a fundamental appreciation for the environment in which they will ultimately be deployed. Research scientists should understand the duty of care that regulators and government have (based on legal regulations) in order to develop tests that meet regulators' data requirements. Regulators should be engaged in developing assays to ensure that they have a fundamental appreciation for the underlying science and are comfortable with the data that are generated. A transparent peer-review process would remain an integral and necessary component of the test development and regulatory acceptance process (Figure 4.10). This holistic approach would permit consideration of the needs of the regulatory community and the development of scientifically credible, fit-for-purpose tests that are based on mechanistically defined endpoints.

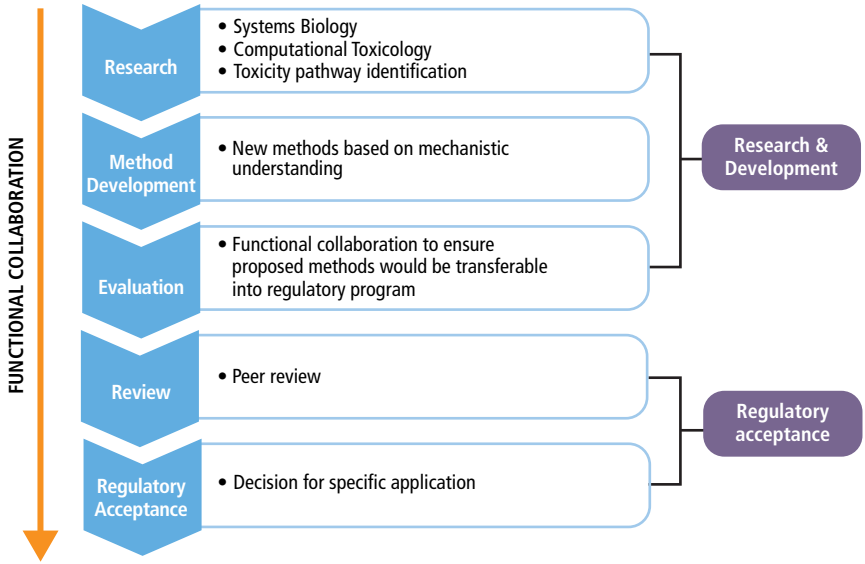

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Figure 4.10

Mapping the process from fundamental research to regulatory acceptance

Any alternative or new method must be developed via a functional collaboration between scientists and regulators to ensure that the methods meet the needs of the regulatory process. Furthermore, an evaluation and peer review of the assumptions, relevance, reliability, sensitivity, and specificity of advanced high-throughput molecular screening and computational profiling methods must occur prior to regulatory acceptance. There should also be an opportunity for public and stakeholder participation and comment.

In this model (Figure 4.10), the reliability of the test describes the reproducibility of its results. Reliability can be addressed by adopting well-designed controls to identify (and minimize the influence of) any confounding variables. Furthermore, the peer-review process would provide an excellent forum for transparent scrutiny of the rationale for a new test, which would reinforce the scientific rigour of test development.

Relevance is equivalent to the utility of the data with respect to their intended purpose (i.e., applicability to regulatory decision-making and the risk assessment context). It is a critical component in developing any test for the regulatory environment.

The data produced in these assays must be useful to the regulatory decision-making process. In the context of screening tools, this regulatory decision may require (or waive) additional toxicity testing. For those chemicals that are currently data-poor,

relevance may be the availability of sufficient data with which to develop rational hypotheses and establish the plausible toxicological potential of a compound or group of compounds. In the context of specific toxicity endpoints, the data may be used to inform a risk assessment and ultimately a registration decision. For data-rich chemicals, relevance in this regard may take longer and will be predicated on building and establishing trust in the new and novel methods.

4.3.3 From Screening Approaches to Toxicity Testing Tools

Before HTS assays can transition from a screening tool to replacing *in vivo* toxicity tests, the quantitative and qualitative linkages between observed cellular perturbations and adverse health outcomes must be established. This relationship would provide the fundamental basis for the development of scientifically robust toxicity tests that can identify and measure those cellular changes that are good indicators of adverse effects at the level of the whole organism.

Any alternative approach would need to be based on the MoA or AOP in humans and yield dose-response predictions for use in setting exposure levels. Such an approach should also be at least as protective as the one it is replacing without imposing unnecessarily strict limitations on chemical usage (R. S. Judson *et al.*, 2011).

Models based on AOP/MoA information that extrapolate *in vitro* data to the *in vivo* situation and take into account bioavailability, clearance, and exposure need to be developed (Blaauboer, 2010). Although so-called reverse pharmacokinetic approaches are in their infancy, considerable progress has been made and proof-of-concept studies are underway. These models could be used to estimate a biological pathway-altering dose (BPAD), which acts as an *in vitro* analogue of an *in vivo* point of departure dose. By incorporating uncertainty and variability into the model, this information could be used to derive the lower confidence bound biological pathway-altering dose (BPADL) via a process of high-throughput risk assessment (HTRA) (Box 4.12).

In the short term, an HTRA approach may be useful in prioritizing data-poor chemicals for subsequent testing; however, its utility to the quantitative risk assessment of data-rich chemicals is predicated on a more comprehensive understanding of the relationship between adaptive and adverse responses at the cellular level.

Fundamental to using any enhanced or augmented IATA is the elucidation of adverse outcome pathways or AOPs. AOPs can causally relate key events at different levels of biological organization to the *in vivo* toxicological endpoint of regulatory interest. They may help clarify the distinctions between adaptive and adverse responses, thus predicting an adverse outcome at the organismal level based on the nature and extent of perturbations at the cellular level. The

Box 4.12
CASE STUDY: Reverse Toxicokinetics and High-Throughput Risk Assessment

High-throughput risk assessment (HTRA) describes a framework by which *in vitro* assay data may be incorporated into existing risk assessment processes (R. S. Judson *et al*., 2011). This framework uses reverse pharmacokinetics studies — designed to interpret the relevance of an effective concentration (in an *in vitro* assay) to human exposure (Rotroff *et al*., 2010) — and incorporates uncertainty and variability in order to derive a biological pathway-altering dose (BPAD) from *in vitro* assay data (Figure 4.11).

Reverse toxicokinetics uses high-throughput assays to look at *in vitro* endpoints (e.g., cellular effects, rates of chemical metabolism, plasma binding levels) and computational tools to extrapolate the *in vitro* data to the *in vivo* system (Rotroff *et al*., 2010). This may be done by calculating the amount of the chemical an individual would need to ingest to achieve a steady-state plasma concentration equivalent to the half-maximal activity concentration (AC_{50}) or lowest effective concentration (LEC) calculated *in vitro* from MoA-based HTS assays.[136]

In order to be useful in a risk assessment, this estimated dose must take into account factors related to experimental uncertainty and population variability. By incorporating uncertainty and variability into the pharmacodynamics and pharmacokinetics analyses, it becomes possible to derive a probability distribution for the pathway-altering dose. The resulting value, the BPADL, represents a permissible exposure level accounting for uncertainty and population variability. The centre of this distribution would be analogous to the No Effect Level (NEL) divided by safety factors. In order to subsequently derive the NOAEL needed for human health risk assessment, it is necessary to determine whether a pathway perturbation is adverse. Discriminating between adaptive and adverse cellular responses is not a trivial task and will necessitate considerable research and policy development.

establishment of a library of AOPs would permit the development of diagnostic *in silico* and *in vitro* models to efficiently measure or profile perturbations (i.e., key events) in normal cellular pathways. This would permit using existing knowledge and AOP information to predict toxicologically relevant outcomes of untested substances, or in read-across to other chemicals.

136 AC_{50} is defined as the concentration that alters the activity of the target in the assay (either positively or negatively) by 50 per cent.

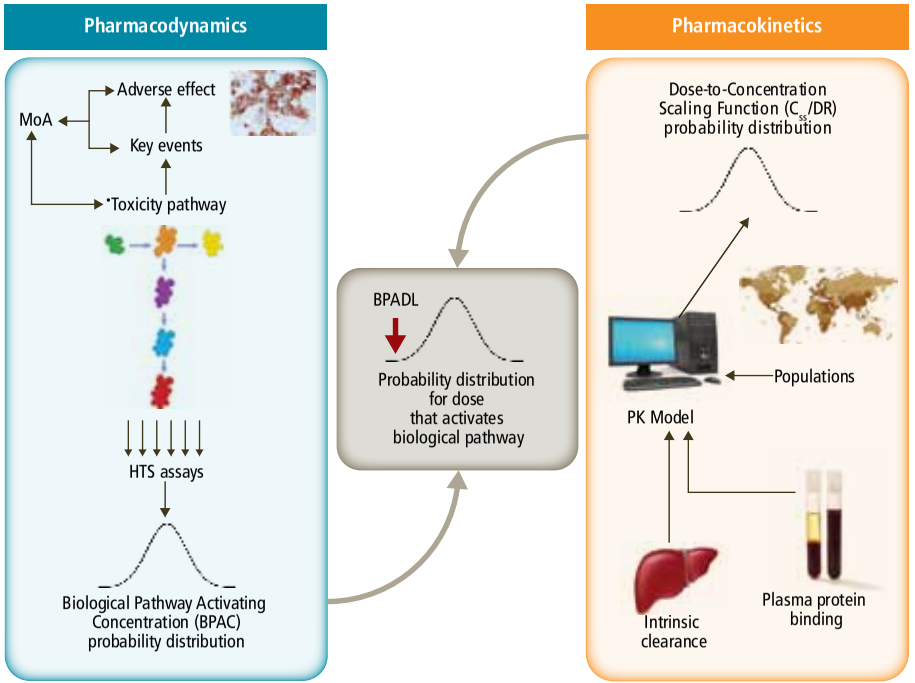

Figure 4.11

High-Throughput Risk Assessment uses Pharmacokinetics and Pharmacodynamics to estimate a permissible exposure level from *in vitro* data

These *in vitro* data are generated using MoA-based HTS assays that assess *in vitro* concentration-response relationships in order to derive a biological pathway-activating concentration (BPAC). By incorporating parameters to account for experimental uncertainty and population variability, HTRA may be a means of deriving conservative exposure limits for data-poor environmental chemicals.

Although this report largely focuses on more effectively filling data gaps for data-poor compounds, the chemical and biological understanding of an AOP could be used for any substance. Discriminating between an adverse and an adaptive response — and developing an understanding of the relationship between the magnitude of a perturbation and the adverse outcome it relates to — could permit the quantitative use of AOPs in decision-making. As a result, the Panel believes that using IATA, when grounded in the knowledge of an AOP, could lead to a more efficient testing strategy for all chemicals, allowing the allocation of finite resources towards those chemicals and endpoints of greatest concern.

4.4 CHAPTER SUMMARY

What is the current status of the use of integrated testing strategies for the risk assessment of pesticides, pharmaceuticals, industrial chemicals, and other chemical substances by regulatory agencies around the world?

There are a number of examples of the use of components of Integrated Approaches to Testing and Assessment (IATA) in a regulatory context for industrial chemicals and personal care products; however, there is no single example of a comprehensive hierarchical deployment of IATA in a regulatory context.

The current approach to regulatory testing has served the needs of risk assessors for many years and has generally been protective of human health. The tests were state of the art at the time of their inception; however, the science has since progressed considerably. New and emerging tools are moving toxicology away from asking *what* and towards explaining *how*. In the long term, the Panel anticipates the adoption of these emerging tools in the regulatory environment will permit a transition away from prescribed data requirements and focus attention on knowledge requirements. This will permit a more hypothesis-driven approach to toxicity testing in which testing resources can be focused on the chemicals and endpoints of concern. In the short term, advances to the use of IATA will likely be realized in their application to data-poor chemicals; however, the use of IATA, when grounded in the knowledge of an adverse outcome pathway (AOP), could lead to a more efficient testing strategy for all chemicals, allowing the allocation of finite resources towards those chemicals and endpoints of greatest concern.

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CHAPTER SUMMARY *(continued)*

The current risk assessment processes are based on the types of data that have historically been generated by toxicity testing. Each of these processes has different data needs so the applicability and relevance of data from alternative testing tools will differ depending on the regulatory activity in question. In particular, the acceptability and applicability of alternative approaches is likely to vary considerably between data-rich and data-poor chemicals.

The quantitative risk assessment process for data-rich chemicals ultimately relies on calculating a numerical value, which means that the data generated from alternative tests do not (necessarily) meet the needs of risk assessors. As a result, although *in vitro*, *in silico*, and omics data may provide mechanistic insight and enhance the interpretation of traditional *in vivo* toxicological data, the use of alternative test methods to quantify risks and establish regulatory endpoints and exposures will remain a challenge. The Panel believes that, over the next decade, *in vitro* assays will see regulatory use for those data-rich chemicals with reasonably well characterized modes of action (MoAs) and for endpoints that cannot be easily assessed using *in vivo* tests. This is already apparent in their adoption for the rapid prioritization of high production volume (HPV) chemicals and the evaluation of endocrine disruption potential.

Legislative reform initiatives to address the lack of primary toxicity data for data-poor industrial chemicals are gaining momentum. The testing and data requirements mandated by these initiatives are unlikely to be met using the existing testing approaches and will necessitate the rapid development and deployment of alternative methods. Long-term solutions to meeting these data needs will not be realized simply by generating more hazard data more quickly. Rather, efficiency (and safety) gains will be made by considering exposure and risk scenarios as well as all existing data in order to target testing to the endpoints of concern. This will necessitate using all available exposure and hazard data, tools, and models. Indeed, the data generated by integrated approaches — including non-animal methods such as (quantitative) structure-activity relationship ((Q)SAR), category formation, read-across, and *in vitro* assays — have historically played a role in priority-setting and screening to determine follow-up actions (or additional testing needs). These tools are expected to continue to evolve in a manner that would provide predictions that are more accurate as the deployment of HTS for the rapid screening of data-poor chemicals would generate a large amount of primary toxicity data and contribute considerably to the understanding of the inherent toxicological properties of numerous environmental chemicals.

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CHAPTER SUMMARY *(continued)*

The dynamic nature of IATA necessitates a new approach to test development, validation, and regulatory acceptance. Test development should be predicated on a functional collaboration between regulators and scientists to ensure that tests evolve to fit the needs of the testing paradigm. This should be coupled with capacity-building initiatives within the regulatory community to build comfort with the science underpinning the alternative tests and to increase familiarity with the data that these tests produce. Alternative tests should be assessed using performance-based standards that judge the utility of a test against knowledge of the underlying biology. These test methods typically target specific cellular or physiological responses and, as such, preclude validation with *in vivo* data by a one-for-one approach. The AOP allows for the use of a suite of models or assays that are designed to target particular steps along a specific pathway. The scientific justification of an alternative method or data set should therefore focus on comparing the test outcome to what is known about the underlying biology as described in the AOP. Therefore, the scientific validation of an alternative test method would be based on mechanistic endpoints that would be measured in assays designed to evaluate a specific cellular or physiological response.

The current approach to testing is entrenched because it is familiar to industry and regulators. This is especially true for heavily regulated, internationally harmonized, data-rich chemicals such as pesticide active ingredients where changes in the data requirements would require international coordination and agreement. The widespread implementation of IATA tools for the evaluation of these chemicals will necessitate the negotiation of scientific, political, practical, legal, and psychological hurdles. The regulation, implementation, and use of alternative test methods, as well as the assimilation of a continuing stream of new data into testing regimes must be harmonized worldwide. Nonetheless, the purpose of regulatory toxicity testing for all chemicals — data-rich and data-poor — is to protect both human health and the environment. The ethical obligation of governments to ensure that regulatory decisions are made using the best available scientific information means that legislative reform and ethical obligation represent extremely powerful drivers of change and innovation in regulatory toxicology.

The utility of IATA is rooted in the elucidation of biological mechanisms that explain toxicological effects. IATA necessitates a dynamic approach that will continue to evolve, and in turn, expand its applicability to the regulatory context as the state of science continues to advance. For this reason it is impossible to predict precisely what

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CHAPTER SUMMARY *(continued)*

the long-term vision of an IATA approach to regulatory toxicology may look like, but it necessitates a more agile, responsive, and mechanism-based testing approach that can exploit state-of-the-art techniques. Implementing any regulatory changes will necessitate a number of scientific and policy challenges, but these will also come with a number of opportunities. The successful implementation of IATA will require a concerted effort by and sustained dialogue between all stakeholder groups including scientists, regulators, policy-makers, and the public.

5

Potential Impacts on the Public's Perception and Confidence in Regulatory Risk Assessment

- The Perception of Acceptable Risk
- Implications for the Adoption of IATA Tools for the Evaluation of Pesticide-Related Risks
- Communications Issues in the Context of Chemical Risk Management

5 Potential Impacts on the Public's Perception and Confidence in Regulatory Risk Assessment

Could there be Potential Impacts on the Public's Perception and Confidence in Regulatory Risk Assessment and Risk Management Decisions for Pesticides if Integrated Testing Strategies were Implemented?

LIST OF KEY TERMS*
Benchmark:
A standard against which something can be judged. In the case of regulatory toxicology, the existing risk assessment practices and safety standards provide the benchmark against which new tests will be judged.
Dread Risk:
A risk of harm or adverse effects that invoke particularly high levels of negative emotion, fear, or even terror.
Risk Communication:
A reciprocal process based on an interactive dialogue among all stakeholders affected by a particular risk.
Risk Perception:
A subjective judgment regarding the characteristics, severity, and acceptability of a risk.

*Key terms as used by the Panel throughout this report. Additional terms are listed in the Technical Glossary in Appendix A.

Changes in the procedures or tools used to inform regulatory decisions related to health and environmental safety nearly always elicit questions and debate. Producers may be concerned about unjustifiably complex, time-consuming, expensive, and over-protective regulatory processes. In contrast, public interest groups may be concerned that changes will compromise the protection of human and environmental health. The common theme throughout is whether the new approaches will enhance or reduce safety; will they increase, decrease, or maintain the current levels and types of risk associated with new chemical products?

The question of what makes risk acceptable in society is highly contested and involves numerous complicating factors. As a result, it can be difficult to achieve social consensus on many risk-related issues. Stakeholders may view the trade-offs between the risk and benefit differently depending on their perspective in relation to the product or activity in question. If a stakeholder stands to benefit from the production and use of a product while others bear the risks, higher levels of risk may seem quite reasonable for that stakeholder. On the other hand, if a stakeholder perceives exposure to the risk as bringing few, if any, benefits, it is highly likely

that much lower levels of the risk will be acceptable (Shrader-Frechette, 1991). The acceptability of new tools for the regulatory risk assessment of pesticides (and other chemical products) is therefore likely to differ depending on the perspective of a given stakeholder group. As a result, the adoption of new testing tools may raise contradictory concerns among stakeholder groups.

Risk perception is affected by a complex mix of personal values, ethics, and conceptual frameworks (Slovic, 1987). As such, establishing the range of potential effects of a regulatory change is not an easy task and necessitates an evaluation that extends well beyond considering the trade-off between risks and benefits. This discussion will form the basis for the subsequent sections.

5.1 THE PERCEPTION OF ACCEPTABLE RISK

There are many examples of how anticipation of and reactions to a hazard can be more important than the simple calculated magnitude of the adverse outcome. Lack of information on the full nature of a risk, coupled with extensive media coverage, can amplify public concerns (Burns *et al.*, 1990; Kasperson *et al.*, 1988). The resulting impact on perceptions may be considerable (Bassil *et al.*, 2007; Chalmers & Jackson, 1996; Sanborn *et al.*, 2007). Addressing risks in a way that is consistent with contemporary views and needs is indeed one of the most challenging tasks of risk management. It necessitates anticipating how the ever-changing nature of perception — affected by new technologies, social pressures, economic factors, political climate, and evolving circumstances such as climate change — can affect risk acceptability at the population level. This, in turn, has a tremendous impact on what may be needed to manage these risks.

An often overlooked, but critical, aspect of risk management is what Leiss and Chociolko (1994) call "managing the risk perception." The way in which a risk is characterized, and subsequently managed, is critical to the way it will be perceived and tolerated by the public. As a result, failure to consider the factors that are known to influence public attitudes towards risk can undermine what seems, from a scientific point of view, to be a reasonable way to manage it.

In the context of Integrated Approaches to Testing and Assessment (IATA), risk management requires consideration, not only of the quantitative toxicity data — together with the inherent uncertainties discussed in Chapter 2 — but also the public's perception of the potential risk posed by changes to the regulatory framework. Experts tend to think of risk and safety in terms of those aspects that can be assessed scientifically (e.g., the magnitude of the hazard, the probabilities of exposure to that hazard, and the balance of the risks against other measurable risks and benefits); however, most non-experts — and even the experts when they

are managing their day-to-day lives — take far more than just these quantitative factors into account when they assess the *acceptability* of a risk. Public perception may be affected by numerous factors. As a result, the acceptability of a risk is quite different from its mere magnitude and depends on a complex array of other, largely qualitative, factors (Krimsky & Golding, 1992).

Individuals are not all inherently risk-averse. Indeed, many people voluntarily expose themselves to high-risk activities (e.g., smoking and extreme sports). The decision to participate in these activities necessitates a personal judgment that balances the perceived benefits against potential risks; rational assessments of probabilities and mathematical calculations are not usually part of that process. Indeed, for many people it is conceivable that, in addition to other perceived benefits of participation, the risk of harm is itself experienced as positive. This is not to imply that the potential harm itself is good (that would be a contradiction in terms) but only that the *risk* of the harm (not the same thing as the harm itself) is intrinsically entwined with the benefits (Kasperson, 1983). This might explain why the voluntarily assumed risks posed by "extreme sports" are attractive to some people.

5.1.1 Critical Factors Influencing the Perception of Acceptable Risk

Covello (1983, 1992) identified nearly 50 factors that influence the perception of risk acceptability. The factors that affect the perception of risk posed by chemical substances are summarized in Box 5.1 and will be explored in more detail in the following subsections.

Box 5.1
A Summary of Key Factors that Affect Public Perceptions of Acceptability for Chemical Risks

- The distribution of risks and benefits is more important than the balance of risks and benefits.
- Unfamiliar risks are less acceptable than those considered to be familiar.
- Hazards that invoke dread are perceived more negatively, even when the risk level is low.
- Risk that is voluntarily taken is more acceptable than a risk that is imposed.
- Risks that people feel they can control are more acceptable than those they cannot.
- Risks imposed by unethical actions are perceived negatively.
- Anthropogenic risk is generally less tolerable than "natural" risk.
- Relative risk is more significant than absolute risk.
- Trust in the risk manager is critical.

Is the Distribution of Risks and Benefits More Important than the Balance?

The risk/benefit approach to determining the acceptability of risks involves weighing the assessed risks against either the benefits that stand to be gained or against the other risks that stand to be reduced. If the benefits or the alternative risks are greater than the magnitude of the risk at hand then, from a purely quantitative risk/benefit perspective, it would seem that the risk in question is acceptable. This method is particularly attractive to a science-based risk management regime because it promises to provide a quantitative algorithm for determining risk acceptability; has the earmarks of scientific objectivity; offers a value-neutral basis for public policy on risk and safety; and helps avoid the subjective and emotional value judgments that influence public perceptions of risk. This approach also comports well with dominant models of economic decision-making, which focus on costs and benefits in a similar way. This commitment to risk/benefit standards of safety explains the tendency of many experts to dismiss non-expert public perceptions of risk and safety as too subjective to meaningfully contribute to regulatory decision-making.

Many value theorists have pointed out that this algorithm is perfectly reasonable when the party standing to benefit is also the one bearing the risks, as is usually the case in financial risk assessment or in the calculation of actuarial risks; however, it is neither reasonable nor ethically appropriate if all parties are not the same (for example, see Rescher, 1983; Shrader-Frechette, 1991). In essence, this means that an individual who bears the risk of an activity, but who does not benefit from it, will perceive that risk very differently than one who bears it but also stands to benefit from it.

Risk management almost always involves cases where the benefits and risks are not evenly distributed among the same parties. It is rare that those who might be most exposed to the risk of harm from a product are those who are, or believe they are, the beneficiaries of that product. As a result, it is rarely persuasive to argue that the benefits of permitting some level of risk from a product outweigh the risks posed by that product. When certain products pose risks to specific vulnerable persons or groups (e.g., infants, the elderly, pregnant women, etc.) or to groups who perceive little benefit from the product, it is not surprising that these groups (and those who represent them) view the risks of the product, however minimal, as unacceptable. A risk/benefit rationale that fails to take the issue of distribution into consideration is therefore unlikely to be persuasive.

Is the Risk Familiar, Unfamiliar, or Uncertain?

Most people are more willing to accept familiar risks than unfamiliar ones. This is usually understood as part of the more general human propensity to fear

the unknown. The Ellsberg paradox has been used to illustrate that people are averse to ambiguous situations where they personally have to estimate the risk (Ellsberg, 1961). The unfamiliarity factor is highly relevant to the impact that scientific uncertainty has on public attitudes towards risk (Tacke, 1999). This partly explains the demand for "precautionary" approaches to evaluating risks which, while deemed small, are often assessed using highly uncertain science.

Does the Hazard Invoke Dread?

Risk-perception research indicates that certain hazards seem to be especially dreaded even when the associated risks are low (Slovic *et al.*, 1982). For example, for most people, the risk of illness or death is not itself as significant as the *type* of illness or the *way* in which they die. Death by cancer is far more dreaded than death by auto accident or pneumonia. These factors cannot always be explained in terms of the probability of the hazard occurring or even of the magnitude of measurable harms (e.g., pain) (Lichtenstein *et al.*, 1978; Slovic *et al.*, 2002).

Is the Risk Voluntary or Involuntary?

A risk that is undertaken voluntarily is typically more acceptable than one perceived to be imposed. Furthermore, research suggests that appreciation of risk depends on how choice is presented (Tversky & Kahneman, 1981). This sentiment reflects a moral value that no individual has the right to impose harm on another without the latter's consent, regardless of the magnitude of the harm. For this reason, risks that are judged to be imposed without consent are not likely to be viewed as acceptable, even when those risks may be very low.

Can the Risk be Controlled?

For reasons closely related to the unfamiliarity factor above, the less control people feel over a risk, the less willing they are to accept it. This partly explains why people engage in high-risk activities, such as automobile transportation; they trust their own ability to avoid accidents. A risk scenario that involves total loss of control or possibility of remediation or a catastrophe, however small, is therefore likely to be judged unacceptable (Covello, 1983, 1992).

Are the Risks Imposed by Ethical or Unethical Activity?

If a risk is perceived to result from unethical actions or motivations, the risk will likely be far less acceptable. The risk of known harm that was unethically covered up or misrepresented is less acceptable for that reason alone (Brunk, 2004). For example, the strong public reaction to the risk of variant Creutzfeldt-Jakob (vCJD) disease during the bovine spongiform encephalopathy (BSE) crisis in the UK, coupled with widespread public discussion that BSE was a result of the "unnatural"

(and therefore unethical) feeding of ruminant protein to (vegetarian) ruminants, resulted in a Europe-wide demand for expensive risk-reduction measures — despite the low incidence of vCJD in humans (BSE Inquiry, 2000; Leiss & Powell, 2004). This was further exacerbated by a perceived failure of government to provide information to consumers that was relevant to their concerns (Frewer, 1999) and to present risk information in a crisis context following a period of perceived complacency regarding the potential human health consequences of BSE (Frewer & Salter, 2002).

Is the Risk the Result of Human Activity (Anthropogenic) or of "Natural" Processes?

People seem to be less accepting of risks resulting from human activity or decisions than those perceived as a result of the force of nature (although this concept has been reviewed by many, Starr (1969) presents one of the earliest-cited discussions of this phenomenon). In other words, people are more accepting of the actions of "Mother Nature" or "God" than they are of each other. This may be because they believe they have a moral right to expect their neighbours to refrain from harming them but have no such claim against an omnipotent being, or because they recognize that they have no way to coerce compliance from the latter.

This factor may help to explain why the common risk acceptability argument (i.e., a risk that does not exceed the "natural background" of the risk should be acceptable) is often not persuasive. For most, the more salient issue relates to the impact of human activity on the overall risk in the environment. People know that life necessarily involves harm but they do not want that harm to be caused by the irresponsible actions of their neighbours.

What is the Importance of Relative Risk, Absolute Risk, and Benchmarks?

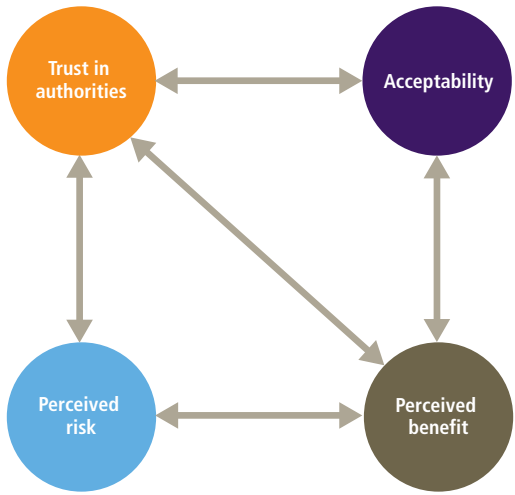
In matters of risk, most people tend to be less concerned about the "absolute" level of a particular risk (e.g., a risk of cancer from a chemical compound) than about how the introduction of that risk, however slight, into their environment changes their risk exposure. In other words, the more salient question is whether the addition of a new product or activity increases their risk relative to the level to which they are accustomed or have come to accept. Indeed, research has shown that most people implicitly establish levels of risk that they consider acceptable, or even prefer, and they compensate for measures that either reduce their risks below that level or increase them beyond it. Wilde (1998) calls this a "risk homeostasis." People who are risk-averse have much lower thresholds for the risk they try to maintain, while those who are risk-takers have much higher thresholds.

This factor goes a long way to explaining the very important role that risk benchmarks play in the perception of acceptable risk. People tend to evaluate the acceptability of some perceived change in their risk environment by referring to a benchmark that represents the level of total risk to which they are accustomed or they find acceptable, according to their own threshold.

Risk experts observe that the general public often demands zero risk and that this demand is irrational given the fact that there is no such thing as zero risk (Grove-White *et al.*, 2000; Marchant, 2001; Marris, 2001). However, what often appears to risk experts as a desire for zero risk in popular opposition to new technologies is, on closer inspection, a demand for no increased risk beyond the benchmark that the public considers acceptable.

Is the Risk Manager Trustworthy?

Of all the factors influencing public perception of risk acceptability, one of the most significant is the perceived trustworthiness of the risk manager (or the risk management regime) (Bronfman *et al.*, 2009; Siegrist *et al.*, 2000).



(Adapted and reproduced with permission from *Safety Science*)*

Figure 5.1

Public risk judgment is impacted by the influence of four core variables

*Reproduced from *Safety Science*, 47/5, Nicolás C. Bronfman, Esperanza López Vázquez, Gabriel Dorantes, An empirical study for the direct and indirect links between trust in regulatory institutions and acceptability of hazards, 686-692, Copyright (2009), with permission from Elsevier.

The public often have limited ability to assess and control risks posed by new technologies. Instead, they must rely on experts to do this for them. Particularly in areas that are scientifically complex, there is a degree of deference to scientific experts and authority, even in relation to controversial topics (Brossard & Nisbet, 2006). As noted above, risks based on assessments that carry a high degree of confidence are often more acceptable than those that carry significant uncertainty.

The public's willingness to accept risks is dependent upon their level of trust in risk experts (i.e., assessors and managers) and the risk assessments these experts conduct. There are many factors that foster this trust, but one of the most significant is the belief that risk managers understand and share the concerns and values of those who rely upon them (Siegrist *et al.*, 2000). There are many ways in which this trust in risk experts may be eroded. The most obvious is when the assurances they give to the public that feared harms will not occur turn out to be wrong. Another way is when the rationale for acceptance of risks fails to reflect the values and concerns of those whose trust is needed.

When the level of trust in experts is high, acceptability of risks also tends to be high; when trust in experts is eroded, the willingness to accept risk may be diminished. Although experts claim that the risk may be insignificant, this claim itself will not be credible (Brunk, 2004). Trust in experts is therefore fragile; the impact of a single adverse event previously characterized as "highly improbable" can undermine the credibility of experts. There are many examples of perceived regulatory failures to manage health or environmental risks that have radically changed the public's willingness to accept levels of risk that are relatively low. For example, the loss of public trust in British regulators after a series of agricultural health crises that they failed to predict or manage (e.g., the outbreaks of Foot and Mouth disease and BSE). This case is often cited as a major contributor to the public perception that the low risk of vCJD is still unacceptable (Lanska, 1998).

5.1.2 The Importance of Factors Impacting Risk Perception

While there is general consensus around the factors described above, there is no agreement on whether these factors are "irrational" elements of risk perception, leading people to make unjustifiable judgments about the actual levels of risk or of risk acceptability, or whether they involve unavoidable and often reasonable value assumptions in judgments about risk and safety (Douglas & Wildavsky, 1982; Gardner, 2008; Shrader-Frechette, 1991; Wynne, 1987). The fact remains that, whether rational or irrational, it is perception that determines what is acceptable. Perceptions, therefore, need to be accorded great weight by public agencies with mandates to regulate and approve chemical products. The regulatory agency that ignores this in its procedures and standards does so at its political peril, since the

widespread view that human or environmental health is not being adequately protected will undermine public trust. If the regulatory decisions are defended as science-based, public faith in science may also be harmed in that process.

5.2 IMPLICATIONS FOR THE ADOPTION OF IATA TOOLS FOR THE EVALUATION OF PESTICIDE-RELATED RISKS

The Panel was asked to consider the potential impact of any changes on the public's perception and confidence in the risk assessment and risk management of pesticides. The Panel considered the aspects of chemical pesticide risks that invoke the perception factors discussed in the preceding section. They also considered how any changes to the regulatory regime — such as implementing IATA tools assessed in this report — might be expected to impinge upon these perceptions.

Chemical pesticide use is a matter of intense concern for certain sectors of the Canadian population (OCFP, 2004). This is evidenced by, among other things, the introduction of numerous local bans on the use of chemical pesticides for cosmetic purposes (reviewed in Box 2.1). Those public interest and advocacy groups that are educated and concerned about the regulation of chemical pesticides can be expected to closely follow any proposed changes to the regulatory framework.

Furthermore, it is important to note that the potentially conflicting opinions held by the public and experts should both be considered equally legitimate. The fundamentally different values upon which these conflicting opinions are based will make it almost impossible to remedy this division. As a result, it is important to consider that failure to effectively communicate the value and purpose of any changes to the regulatory system may result in an information vacuum. This vacuum will readily be filled by messages from advocacy groups that communicate their own interpretations of the implications for public safety to the communities that they represent (Leiss & Powell, 2004). Consequently, the ways in which these tools are used, and the changes they make in the risk management regime, need to be thought through carefully with a view to the way they will be interpreted publicly and the impact this can have upon public perception of the risks associated with chemical pesticide products.

5.2.1 A Brief Review of the Panel's Assessment of IATA Tools

As discussed in the preceding chapters, the issues inherent in the current approach are two-fold. There is a need to address the lack of toxicity data for the vast majority of industrial chemicals, as well as to recognize that regulatory decisions must be made based on the best available science. The Panel believes that adopting alternative approaches in regulatory toxicity testing can address some of the

limitations in the existing approach and significantly enhance the assessment and management of the risks of chemical compounds in ways that serve human and environmental safety as well as the interests of other stakeholders.

An IATA approach seeks to integrate all useful data to inform a risk assessment via a hierarchical approach to testing (Figure 4.1). It adopts and integrates new and emerging tools that could move toxicology away from asking *what* and towards explaining *how*. In turn, this could help to reduce the uncertainties present in the existing approach.

Chapter 4 described how *in silico* IATA tools have already been extensively used to support regulatory decision-making for priority setting of data-poor chemicals. The Panel anticipates that *in vitro* high-throughput screening (HTS) assays will be adopted in the short-term to facilitate the rapid generation of primary data for these data-poor chemicals, which will enhance the capacity of the regulatory system to protect human and environmental health.

Currently, no set of alternative methods can replace the entire testing paradigm for data-rich chemicals; however, the Panel anticipates that alternative approaches for evaluating acute toxicity endpoints and critical local effects could be in regulatory use in the very near future. As the state of the science evolves, the Panel also anticipates that augmenting the existing procedures will become increasingly feasible. In the long term (more than 10 years), as alternative tools are developed and refined, the Panel anticipates that the regulatory approach for all chemicals could transition to one that screens and evaluates all chemicals in order to focus testing resources on the endpoints and on chemicals of concern.

5.2.2 The Profile of Chemical Pesticide Risks and Risk Management

The aspects of public risk perception summarized in Section 5.1 represent useful tools to predict the impact of IATA adoption on public perception. Using these tools, it is possible to determine the kinds of implementations that would likely trigger different concerns and responses. A helpful way to do this is to understand the general "profile" of chemical pesticide risk and its management. It is then easier to see how different changes to the risk assessment and management system would likely influence perceptions.

Distribution of Chemical Risks and Benefits:

Risks and benefits associated with a product, even one as strictly regulated as a pesticide, are not equally distributed across various population groups and the environment. For this reason the question of "Who enjoys the benefits, and who

bears the risks?" becomes critical to the acceptability of the risk. As discussed in Section 5.1, few are willing to accept risks (however small) that are imposed on them by others for the others' benefit.

Chemical pesticides are complex in this regard because they have been widely used in agricultural, public recreational, and private residential contexts. As a result, there are many people who might legitimately see themselves as benefiting from them, either directly or indirectly. Many homeowners have used pesticides regularly on lawns, gardens, and houseplants. Nonetheless, the risk/benefit equation seems to be shifting in the public mind as part of a growing environmental consciousness. It appears that more people are coming to view themselves (and particularly their children) as primarily bearing the risk of pesticide use (whatever this may be) while not receiving significant benefits. This shift in perception of the risk/benefit equation is likely a significant factor in the recent ban of residential pesticide sale and use in Ontario and Quebec (reviewed in Chapter 2).

A critical question to address when assessing the perceived acceptability of an IATA approach to pesticide regulation is whose interests are really being served by its adoption. Public health and environmental advocates will want to know the benefits and to whom they will accrue. For example:

- Who would be the primary beneficiary if IATA tools were used to increase the efficiency and speed of the regulatory approval process? Would it be the public, because the process of regulatory approval for safer alternatives is significantly decreased? Or would it be industry, through more expeditious access to the market?
- If IATA tools were used to identify "false positive" adverse outcomes in animal tests and provide scientific justification for the approval of previously excluded chemical pesticides, critics could interpret this as handling scientific uncertainties to benefit the industry at the expense of public safety.
- If IATA tools are used to close the current information gap on data-poor chemicals and provide a rationale for regulatory exclusion from the market of previously approved pesticides, this would provide a counter-argument to the above criticism that the system was compensating by significantly reducing the number of "false negative" regulatory decisions. This would give greater weight to public safety over the industrial benefits.
- If implementing IATA tools can be shown to provide a net increase in the identification of harmful chemical pesticides and a reallocation of limited government resources to those chemicals with a higher regulatory priority, this would be a strong case for increased safety and benefit to the public.

Failure to address these concerns could negatively impact public acceptance of any new testing approaches. Critics would need to be persuaded that these regulatory changes would not redistribute the risks and benefits in ways that could increase risks to some for the benefit of others.

Unfamiliarity and Uncertainty in Toxicity Testing of Chemicals:

While chemical pesticides have become a recognized part of Canadian life in the past half-century, their use has become increasingly controversial. A significant part of this controversy stems from an increased awareness of the uncertainties present in the scientific assessment process. Stakeholders on all sides widely acknowledge and criticize these uncertainties. Awareness of these uncertainties may explain why members of the public typically rate chemical risks as more significant than do most experts (Kraus *et al.*, 1992; Krewski *et al.*, 2008; Slovic *et al.*, 1995). Many of the books, articles, and media reports that incite public concern about the potential impacts of chemical residues in food and in the environment often cite the uncertainties in the science upon which the regulation of these chemicals depend.[137]

The adoption of IATA tools could increase the understanding of the underlying mechanisms of human toxicity and thus reduce uncertainty at this level. Nevertheless, it does introduce a new set of uncertainties (reviewed in Chapter 3). It will be important to be clear that the existing approaches generally handle the uncertainties by building in highly conservative margins of safety. These handle the endemic uncertainties in a highly precautionary way. The Panel believes a significant question regulators will need to address as they implement new IATA tools is the extent to which they reduce the uncertainties that would justify the use of less conservative margins of safety. Such an approach would raise questions among some stakeholders about whether the new tools sufficiently reduce overall uncertainties to justify adopting less conservative safety margins. A very strong case would need to be made for the increased scientific reliability of these new tools. If the public perception of any changes is that scientific rigour and precautionary assumptions are being sacrificed in the interest of greater economic and regulatory efficiency, this could easily result in lowered confidence in the system of chemical pesticide regulation on the part of significant sectors of the public.

The issues of unfamiliarity and uncertainty are at the centre of the discussions around precaution and the precautionary principle. The precautionary principle is commonly used as a rule (or set of rules) for handling uncertainties in science. We only need to observe that the greater the perception of uncertainty around

137 See for example, *Slow Death by Rubber Duck* by Smith & Lourie (2009).

risks of any technology, the greater the demand for precaution will be, at least on the side of the stakeholders who view themselves primarily as the risk bearers. This issue was discussed in the previous section as it relates to the manner in which handling the uncertainties can be perceived to change the distribution of risks and benefits in society.

The Impact of the Dread Factor in Relation to Chemicals:

In Canada, and North America in general, cancer is the paradigmatic dread disease (Beach *et al.*, 2005; Clarke & Everest, 2006; Dich *et al.*, 1997; Fife & Wright, 2000).[138] Because of the high dread factor associated with cancer in Canadian society, both industry and regulators recognize that the public is likely to demand higher standards of precaution in the implementation of new, and especially alternative, tools for the assessment of chemical pesticide risks than for many other types of risk. The public demand for the banning of cosmetic pesticides in various provinces of Canada (reviewed in Chapter 2), despite the predominant scientific view that these risks are generally over-estimated (for example, see STATS, 2009), is a graphic example of the power of this attitude towards cancer risks. This is another reason why it is important that these tools be implemented in a way that both enhances, and is seen to enhance, the ability to identify risks to human health. It is also a powerful illustration of how the existence of an information void may generate fear and suspicion among those affected by the risk in question, which serves to reinforce the messages from advocacy groups. This is a concept that will be explored in more detail in Section 5.3.

The Ethical Activity Factor in Chemical Toxicity Testing:

In order to provide exhaustive answers to questions on the state of the science supporting IATA and the status of implementations, the Panel deliberately focused on the scientific merit of adopting IATA rather than social or ethical benefits.[139] Nonetheless, the Panel recognizes the significance of such benefits, in part because socially desirable outcomes help in the adoption and acceptance of new technologies and scientific methods.

An important issue surrounding the adoption of IATA is the expectation that these tools will lead to a significant reduction in animal-based testing. This is important because of the growing ethical sensitivity in Canadian society, as elsewhere, to the use of animals in the kind of research that is required by the *in vivo* paradigm.

138 In June 2008, the Canadian Partnership Against Cancer, a government-funded agency, launched a study (anticipated total investment approaching C$200 million) that will track 300,000 Canadians over decades to explore how genetics, environment, lifestyle, and behaviour contribute to the development of cancer: http://www.partnershipagainstcancer.ca/

139 The impact of alternative and *in vitro* approaches to toxicity testing on animal use have been explored elsewhere (Stephens, 2010).

Animal welfare and animal rights advocates have long pointed out the burden of suffering placed upon animals in order to reduce the risks to ourselves and have also questioned the reliability of the knowledge obtained (e.g., Balls, 1994; Purchase, 1999). Most animal research in Canada is scrutinized by animal research ethics review panels and institutions such as the Canadian Council on Animal Care (CCAC).[140] The application of the 3R principles — Reduce, Replace, and Refine — first espoused by Russell and Burch (1959) to animal testing in regulatory toxicology over 50 years ago are almost universally endorsed. Consequently, changes in the regulatory assessment of chemicals that move away from reliance upon *in vivo* toxicity testing would undoubtedly have a positive impact on public attitudes towards the regulatory system, and hence, towards the acceptability of the risks in the products that are approved for production and sale in Canada.

As discussed earlier, the Panel anticipates that adopting alternative approaches for data-rich chemicals such as pesticides will take many years. As a result, it is unlikely that an IATA strategy would result in a significant reduction in the use of animal studies in the short term. Therefore, accurate communication will be essential to avoid misled expectations. The clarity and consistency of the message detailing the anticipated benefits of the new regulatory tools will be a critical success factor for acceptance of IATA.

The Critical Importance of the Benchmark for the Current Approach:

In Canada, as elsewhere, the current benchmark for acceptable risk load associated with chemical pesticides derives from the perceived level of risks deemed acceptable under the current testing system. As described in Chapter 2, although there are a number of limitations in the existing approach to regulatory testing of chemicals in general, neither risk experts nor the public share unanimous views about the current system. While concerns exist about the capacity of the current system to adequately protect human and environmental health, there are also conflicting concerns regarding the possibility that the system may be too conservative (US FDA, 2004).

Nevertheless, from the perspective of risk acceptability, the current system will provide the benchmark against which any new risk management regime will be judged. Any changes perceived as increasing uncertainty or decreasing the level of health or environmental protection would likely raise significant public concern. On the other hand, changes that would convincingly reduce uncertainty and increase identification of significant risks might be expected to increase (or at least not erode) trust in the management of chemical risks.

140 The Canadian Council on Animal Care: http://www.ccac.ca

Benchmarks and the Need for a Perception Profile:
Understanding the benchmark perception of pesticide-related risks would provide us with an important set of tools to predict the impact that IATA may have on public confidence in the regulatory system. Defining this "perception profile" for pesticides is a difficult task because pesticides are used in a variety of contexts, some of which may be more apparent than others (e.g., agricultural pesticides, parasite control, and residential). Different stakeholder groups will have different views on the acceptability, safety, and risks associated with the use of pesticides in these different contexts.

Studies that evaluate the perceptions of different stakeholder groups are critical to identifying the issues of concern and to developing strategies to address them. Canada is a large country with geographically dispersed populations, which makes collecting reliable and representative benchmark data challenging. Although extensive Canada-wide studies on the public perception of the regulation of pesticide risks are not available, Health Canada has carried out some preliminary work in this area (Box 5.2). This example highlights one of the main difficulties in risk assessment; the need to provide a transparent assessment of the inherent uncertainty while remaining cognizant of the public's desire for unambiguous statements from the regulatory authorities. One can be unambiguous about the nature and levels of uncertainty, and generally, this is a more trustworthy approach as it communicates more certainty than the science supports.

Box 5.2
CASE STUDY: Establishing Benchmarks for the Public Perception of Pesticide Risk

In March 2007 the Public Opinion Research and Evaluation Unit released the findings from 12 focus groups that were conducted on behalf of the PMRA (Strategic Counsel, 2007).

The objectives of the study were:
- to measure the effectiveness and comprehensiveness of commonly used messages in public communication about pesticides; and,
- to measure the readability and effectiveness of label improvements for domestic and commercial class pesticide products.

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Box 5.2 *(continued)*

The focus groups were conducted across Canada with participants from the general population (four groups), opinion leaders (four groups), and farmers (four groups). Some key findings from this study are discussed below.

Perception of Pesticide Regulation (general population and opinion leaders)

- Participants were generally unaware exactly who or what agency was responsible for regulating pesticides (few identified the PMRA, although they expected that there is some dedicated entity).
- Health Canada was generally considered to be highly credible, based on a belief that their scientists are both experts in their field and unbiased in their assessments. In every group however, some questioned the government's integrity in regulating business. They were also wary of governmental assurances of safety (if assessed unsafe, that is true; but if assessed "safe," that is only partly true due to governmental standards on acceptability of risk).
- Although most participants expressed faith in the intentions and integrity of Health Canada, they felt that easier access to information would provide additional reassurance.

Communicating in the Media (general population and opinion leaders)

- Health Canada's expertise lies with its scientists who are viewed as unbiased, neutral, and credible. References to "scientific," "research," or "science-based risk assessments" are reassuring.
- Referring to the specific agency mandated to regulate pesticides (i.e., PMRA) highlights the existence of a group of qualified people dedicated to assessing the risks and determining the safety of pesticides for general public use.
- Highlighting the continuous nature of monitoring and ongoing studies that inform evaluations of pesticide products would bolster confidence.
- The use of active, strong words (e.g., determined, strict, thorough, and health and safety) and definitive phrases is preferable. Directional statements (e.g., "acceptable use") may be open to interpretation, which places the onus and responsibility on the user rather than Health Canada and makes Health Canada look unwilling to take a stand.
- The use of the phrase "available studies" was sometimes interpreted as Health Canada "picking and choosing" their evidence.

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Box 5.2 *(continued)*

- There was some surprise regarding announcements on re-evaluations for pesticides. Many had assumed that modern standards were being applied on an ongoing basis (i.e., that as new information is obtained and standards evolve, all existing products on the market are continuously monitored for conformity).
- The term "re-evaluation" is not necessarily viewed positively because it suggests the public's health and safety may have been previously jeopardized. This is significant given the emphasis on re-evaluation as a key function within the regulatory mandate. Whereas, the term "continuous monitoring" seemed to leave a more positive impression, suggesting that regulators were being proactive rather than reactive.
- Although the small size of the sample population coupled with the qualitative nature of these data preclude a direct extrapolation to the Canadian population as a whole, they do illustrate the value of stakeholder engagement as a means of understanding the issues relevant to different communities.

What would the use of the new and developing IATA tools do to these benchmarks? The new scientific knowledge, methods, and tools (described in Chapter 3) as well as the impact of regulatory implementation (described in Chapter 4) show the drive towards improved reliability of the science upon which risk assessment and regulatory decisions are made.

Research suggests that, for Canadians, the current benchmark of acceptable risk load rests in a fairly high level of trust in the current regulatory system and the science on which this is based (Krewski *et al.*, 2008; Slovic *et al.*, 1995). The replacement of existing tests with alternatives that are not able to provide adequate data to assure the safety and protection of human and environmental health would erode the current benchmark. This, in turn, would significantly erode public confidence in the regulation of chemical risks. Conversely, the use of these tools to decrease uncertainties, and thus increase the level of protection beyond the current benchmark, might even enhance public confidence in the regulatory procedure. One caveat would be that such positive impacts are effectively communicated to stakeholders and the public.

Public Trust in Chemical Risk Management:

Evidence suggests that a trusted regulatory scheme can increase the acceptability of higher levels of risk and uncertainty. As noted by Bronfman *et al.* (2009), the "linear relationship between perceived risk and acceptability is mediated by the extent of social trust and the benefit perceived." The implication for regulators

is that it is important not only to manage risks of concern to the public in an effective manner, but also to be *seen* to manage them effectively and in the public interest; however, balancing the interests of the general public and those of specific stakeholder groups often involves controversies. Trust-building is a long process that can be easily undermined. It is also worth noting that the erosion of public trust can have many roots, most notably the involvement of commercial interests and a failure to address an information void. Given the sensitive nature of the pesticide issue (Coppin *et al.*, 2002), trust in the experts seems likely to be relatively fragile — as it is with biotechnologies in general (Krewski, 2005).

A singularly critical factor in establishing and maintaining public trust in the regulatory system is transparency — in the process and in the underlying rationales for the decisions reached. Consequently, implementing the new integrated testing tools that are the subject of this report needs to be done in an open and transparent manner. In this context, openness and transparency mean:

- clearly communicating, with stakeholders and the public, the steps to be taken in integrating new testing tools into the regulatory risk assessment process and the rationale for these steps; and
- engaging stakeholders and the public in an open discussion of these proposals; addressing their concerns; and incorporating their suggestions into the structure and function of the system.

It is critical, in these discussion forums, to be open and honest about the uncertainties present in both current and proposed scientific tools and to be clear about how these issues will be handled in ways that serve public and stakeholder interests.

Communicating with stakeholders is of paramount importance to avoid information gaps. In December 2010, as part of the US EPA Pesticide program a stakeholder workshop was held on the use of 21st century science and integrated testing and assessment strategies. The aim was to broaden and strengthen stakeholder dialogue and to increase a common understanding of these new science tools and how best to apply them.[141] Of the different stakeholder perspectives expressed at this meeting, it was stressed that regulatory authorities need to build a process for how decisions will be made based on the new science tools and that a system needs to be in place to evaluate the accuracy and effectiveness of decisions (Dellarco, personal communication).

141 Full details of the workshop may be found at: http://www.epa.gov/pesticides/ppdc/testing/index.html

5.3 COMMUNICATIONS ISSUES IN THE CONTEXT OF CHEMICAL RISK MANAGEMENT

Public communication is a central component of risk management, and it will play a major role in the successful implementation of new regulatory policy. It is a theme that cuts through many of the above-noted risk management/perception factors. As such, it is critical to consider some of the most salient science communication issues.

There is a common belief that "misperceptions" or concerns about risks or science-based regulation can be solved by informing the public about the relevant science. The idea is based on the belief that a more informed and scientifically literate public will be more accepting of new technologies and will be less concerned about concomitant risks. This approach, known as the "deficit model," is problematic on a number of levels. There seems little doubt that knowledge can play an important role in attitudes about science. Studies have shown there is a correlation, one that is stable across cultures and domains of scientific inquiry, between positive views of science and knowledge about scientific facts (Allum *et al.*, 2008). Nevertheless, the relationship between the provision of information and acceptance is far from simple. Simply explaining the relevant science will not, on its own, necessarily lead to higher levels of acceptance (Simon, 2010; Sjoberg, 2008; Sturgis & Allum, 2004).

For example, if individuals lose trust in or have reservations about a technology or regulatory approach, they will seek or at least be more open to information that confirms these views (Bubela *et al.*, 2009). If members of the public have concerns about pesticides, they may be drawn to media stories that validate these pre-existing beliefs. Simply disseminating information to the public about the relevant science and rationales for regulatory reform, no matter how scientifically sound, will not necessarily lead to greater public acceptance. Failure to recognize this and appreciate its significance can lead to an information gap between stakeholders (Leiss & Powell, 2004).

The existence of an information gap can have a significant, negative impact on public trust and confidence in the regulatory process because this void will readily be filled by information from other sources. Those sources may not be credible and may rely on data that are selectively chosen and scientifically weak; nevertheless, in the absence of convincing and credible scientific arguments, such information fills a need. Some advocacy groups (and the media) may frame their arguments in a way that resonates with the communities they represent; they selectively choose to present specific data that would frame and promote their cause. Failure to address scientifically questionable claims by the media and other sources can undermine the credibility of the regulators, which in

turn will significantly erode public trust in their ability to protect human and environmental health. Perhaps nowhere was this more evident than the case of the recent emergence of cosmetic pesticide bans across Canada (introduced in Chapter 2). The push to ban the sale and use of cosmetic pesticides in numerous Canadian jurisdictions has largely been attributed to the work of advocacy groups, whose powerful communications campaigns were met by silence from the federal regulatory agencies. This campaign was supported by testimony from medical doctors — the majority of whom have no formal training in the interpretation of toxicological or epidemiological data — whose opinions are considered by many members of the public to be the most trustworthy, and more credible than those of scientists (Box 5.3) (Krewski *et al.*, 2006). This is a powerful example of how the source of the information can be as important as the information itself.

Box 5.3
CASE STUDY: The Role of Medical Health Officers in Cosmetic Pesticide Bans

Medical Officers of Health arguably played a significant role in the introduction of legislation to ban the non-essential use of pesticides for cosmetic purposes in urban settings in Canada. An excellent example of this is Dr. Sheela Basrur, who served as the Medical Officer of Health in Toronto. Her involvement included advising the Toronto Board of Health (TBH) of the ability of a municipality to regulate the non-essential outdoor use of pesticides, based on the Hudson, Quebec experience (Basrur, 2002b). As a result, in 2001 the TBH directed Dr. Basrur to prepare a public discussion document and to obtain wide input into the nature and scope of a potential bylaw. Her report *Playing it Safe: Healthy Choices about Lawn Care Pesticides* (Basrur, 2002a) was prepared by members of the Health Promotion and Environmental Protection Office, Toronto Public Health, and released by Dr. Basrur in April 2002 subsequent to initial public consultation in Toronto. One purpose of this document was to facilitate additional consultation with the public. The authors stated there were enough reports of the potential harmful effects of pesticide exposures in the scientific literature to warrant reduced exposure of children and other vulnerable individuals to chemical pesticides. This disclosure by municipal health professionals probably influenced the outcome of the consultations, which showed that more than two-thirds of those who participated favoured the restricted use of non-essential pesticides. Toronto's Pesticide Bylaw (Municipal Code 612) came into effect on 1 April 2004 and remained in place until the Province of Ontario introduced legislation to ban the cosmetic use of pesticides. Ontario Regulation 63/09 was enacted on 22 April 2009 (Government of Ontario, 2009).

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Box 5.3 *(continued)*

In a similar vein, the Ottawa Board of Health/City Council requested an update on the health effects of pesticides from its then Medical Officer of Health, Dr. Robert Cushman, which he completed in August 2005 (Cushman, 2005). Dr. Cushman noted what had occurred in Toronto and included a literature review of the harmful effects of pesticides, noting reports of increased sensitivity of individuals with specific genetic polymorphisms to pesticides (Elbaz *et al.*, 2004; Infante-Rivard *et al.*, 1999). He concluded in his report that the precautionary principle and existing scientific evidence about the harmful effects of pesticide exposure warranted a bylaw to prohibit the cosmetic use of pesticides in Ottawa (Cushman, 2005). This report was presented to Ottawa City Council by Dr. David Salisbury, the new Medical Health Officer, in October 2005, but it did not precipitate immediate action in terms of a bylaw. In June 2007, one of the Ottawa City Councillors requested an update of the medical literature of pesticide effects. Dr. Salisbury sent his report, which analyzed the literature published between 2005 and 2007, to City Council on 6 September 2007 (Salisbury, 2007). His conclusions reflected those reached earlier by Drs. Basrur and Cushman. In his concluding comments, Dr. Salisbury also referred to the recommendation made in 2007 by Justice Archie Campbell in the Severe Acute Respiratory Syndrome (SARS) Commission final report, an acute event that resulted in the death of 44 individuals in Ontario, three of whom were health-care workers. Justice Campbell noted that the precautionary principle had not been adequately applied during SARS and went on to suggest this principle should be applied throughout the health system in Ontario.

Although Ottawa never enacted its own bylaw banning the cosmetic uses of pesticides it did endorse the initiative of the Ontario Government to ban the sale and use of non-essential pesticide use in May 2008. In summary, it is prudent to note that the activities of Drs. Cushman and Salisbury, Ottawa's Medical Officers of Health during this period, played an influential role in this outcome.

The provision of accurate and balanced information is therefore vitally important (Tyshenko *et al.*, 2008). If the public views the information as accurate and provided by an independent source (especially one that is free of commercial influence), it can help build trust, satisfy the ethical norm of transparency, and can lead to greater comfort with the regulatory approach over time (Krewski, 2005; Krewski *et al.*, 2008).

It should be noted that the popular press plays an important role in this regard. The public gets most of its information about science and health issues from the

news media, the internet, and medical doctors (Krewski *et al.*, 2006). While it is important to avoid overly simplifying the relationship between media representations and public perception (popular culture both reflects and informs public perceptions), there seems little doubt that the media plays an important framing role (Bubela *et al.*, 2009; Clarke & Everest, 2006; Nisbet & Mooney, 2007). That is, they provide the public with the relevant information and frame the public dialogue (e.g., set the story as a negative or positive technological development) (Marks *et al.*, 2007).

Regardless of the scientific accuracy of these representations, failure on the part of regulators to challenge them can have a profound impact on public debate and the subsequent implementation of regulatory policy. As a result, the provision of accurate and balanced information remains vitally important.

Transparency is a critical component in building public confidence in the regulatory system. The use of any new tools must be explained as clearly and accurately as possible, and the approaches for the handling of the changes in scientific certainty and uncertainty must be clear. Indeed, governmental agencies in Canada and other countries are facing increased pressure to operate in an open and transparent manner (Box 5.4). To ensure that regulatory decisions are made in an open and transparent manner (while respecting confidential business information involved in submissions to PMRA from the pesticide industry), it is important that the data requirements for registration of a new product are clearly specified, that the risk assessment criteria used to evaluate such data are explicit, and that the risk management principles that guide regulatory decisions regarding pesticides are clearly articulated. To the extent possible, the data used to support pesticide regulations should be available for re-evaluation by interested parties.[142]

Box 5.4
An Aside on the Future of Openness and Transparency in Canadian Regulations: A TBS Directive

The Treasury Board of Canada Secretariat (TBS) developed a document titled *Guidance on Risk Assessment for Public and Environmental Protection in Federal Departments and Agencies: Regulatory Proposals* in which openness and transparency is identified as a key principle. The Treasury Board guidance to federal governmental departments is as follows:

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142 Subject to legal restrictions pertaining to confidential business information.

Box 5.4 *(continued)*

Information on the objectives, evidence, process, and conclusions of a risk assessment should be made available to governmental partners, stakeholders and the interested public, subject to valid privacy, proprietary information, and security constraints. Risk assessment processes that support important public decisions should include consultation with stakeholders and the public, as appropriate to the nature of the issue and the level and extent of stakeholder interest. Ideally, risk communication continues with interested stakeholders throughout the process. As part of ensuring transparency, the process and criteria used in the consideration of input received during consultation should be described. While the risk assessment process should both accommodate and support openness and transparency, the extent to which this is pursued is typically determined as a matter of risk management.

Current government policies on access to information assume that all information used or generated by government in its decision-making on behalf of the public should be made publicly available unless there are legitimate reasons to protect it. For example, the protection of confidential business information in competitive markets is seen as necessary for a well-functioning and innovative economy. Likewise, requirements of national security and defence may justify the need for a level of confidentiality that would restrict the extent of transparency.

Departments should have procedures in place to ensure openness and transparency while respecting required levels of confidentiality. Transparency, both in evidence and in process, helps stakeholders and Canadians to understand the rationale for government decisions. In certain cases, appropriate application of confidentiality provisions is required to protect proprietary information, or to protect information related to the security of Canada and Canadians.

In a checklist for risk assessment, criteria used to evaluate transparency are based on the following questions:

- Was information on the objectives, evidence, process, and conclusions of the risk assessment made available to governmental partners, stakeholders, and the interested public?
- Were key stakeholders consulted on the risk assessment?
- Were their views and opinions made available to the public?
- Was an expert panel convened or were experts consulted on the risk assessment?
- Was the process and criteria used in the consideration of input received described?

5.4 CHAPTER SUMMARY

Could there be potential impacts on the public's perception and confidence in regulatory risk assessment and risk management decisions for pesticides if integrated testing strategies were implemented?

Yes. A major question that will be raised as a result of implementing Integrated Approaches to Testing and Assessment (IATA) tools in the regulatory system will be whether these changes enhance the ability to identify the most important risks to human health and environment, or whether they compromise this ability in the interest of other social and economic values. The public will likely demand assurances that the new methods reduce overall uncertainties in the assessment of chemical risk, and that, where new uncertainties are introduced, these will be handled in ways that are at least as precautionary as in the current system.

While there is not a high level of public understanding of the strengths and weaknesses of the current system of chemical risk management in Canada, it does constitute a "benchmark" against which changes in the system will likely be evaluated by concerned stakeholders. The questions that regulators would need to address would likely include the following:

- Will the new IATA tools be used to supplement (and thus strengthen) the current system or to replace it?
- What scientific uncertainties in the current system of chemicals management will implementing new IATA tools reduce? What uncertainties will be introduced?
- How will the changes in the scientific uncertainties be handled in the regulatory process? Will the current "margins of safety" used in the *in vivo* toxicity testing regime be reduced? Will this lead to a reduction in the level of precaution exercised with respect to certain kinds of chemicals?

The Panel believes that the new IATA tools should only be introduced into the regulatory system in a supplementary manner. This can be done in such a way as to increase the ability of the system to more reliably identify the most significant risks, especially with respect to data-poor chemicals. If done in this way, those issues of public concern summarized above can be addressed in a way that maintains, and even strengthens, public confidence in the regulation of chemical pesticides.

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CHAPTER SUMMARY *(continued)*

If the Canadian regulatory agencies move towards implementing the IATA tools, it is vital that they also engage in effective strategies to engage stakeholders and the Canadian public in discussions that permit the meaningful consideration of their interests and concerns.

The greater the transparency in the process of implementation, the communication of the rationale for that implementation, and the involvement of the public in that implementation, the greater will be the levels of public trust in these changes. To this end, any change in the regulatory paradigm should be accompanied by sustained, sincere, and transparent dialogue with stakeholder groups, including the general public.

6

Integrating Emerging Technologies into Chemical Safety Assessment

6 Integrating Emerging Technologies into Chemical Safety Assessment

The active ingredients of pesticides are among the most stringently regulated chemicals in commerce; the toxicological assessment of the active ingredient follows a regimen that is similar to that for the preclinical assessment of prescription drugs. Before a pesticide can be registered for sale or use in Canada, risk assessors use the data derived from these tests to evaluate the potential risk to human health and the environment. This extensive evaluation of the active ingredients, however, contrasts with the data requirements for the other components of the final pesticide product. These formulants, which are added to pesticide products to improve their physicochemical properties, enhance their use, or increase their stability, are not typically subject to a stringent battery of toxicity tests and are often data-limited. As a result, the final pesticide product contains a combination of data-rich and data-poor chemicals.

The Panel believes that the data-rich and data-poor nature of a pesticide formulation is a compelling metaphor for the dichotomy that exists for most industrial chemicals. While there are some substances for which an enormous amount of data are available (e.g., pesticide active ingredients and pharmaceutical drugs), the vast majority of industrial chemicals are extremely data-poor.

The current hazard identification approach for data-rich chemicals relies extensively on data derived from observing apical endpoints from animal studies using a suite of standardized protocols. These tests were designed to minimize variance and to provide a robust and comprehensive data set upon which to base subsequent regulatory decisions. Although this approach has served the needs of risk assessors for several decades and is generally believed to have been health-protective, many of the tests have not changed appreciably since their inception over 30 years ago. It is expensive, time-consuming, and cannot adequately evaluate the potential hazards of the large numbers of uncharacterized chemicals that have little or no data.

The issues inherent in the current approach are therefore two-fold: to address the lack of toxicity data for the vast majority of industrial chemicals and to recognize that regulatory decisions must be made on the basis of the best available science. As a result, there is a need for new approaches that are more predictive, more reliable, faster and less expensive, and that provide mechanism-based chemical-specific toxicity information in order to better inform human health risk assessment.

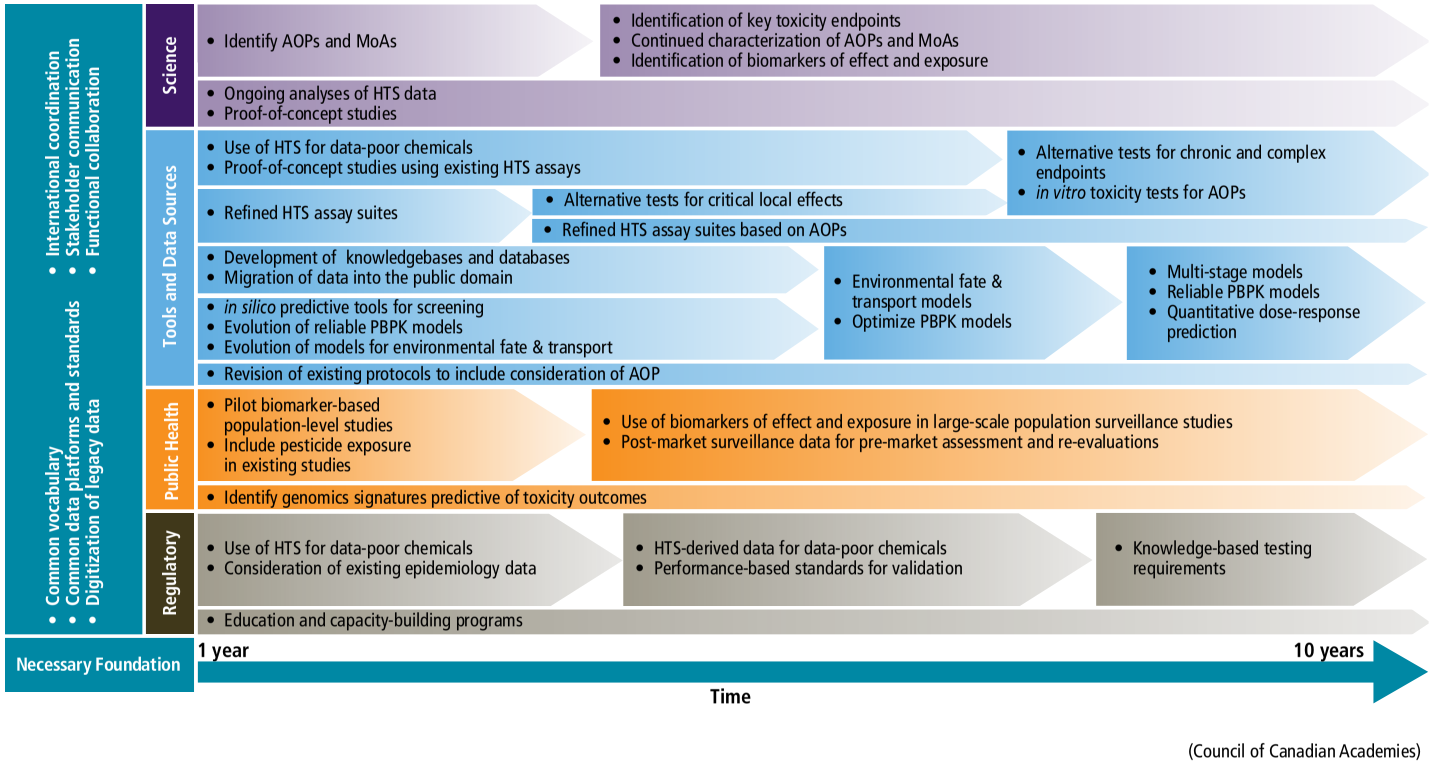
New methods are being developed; these are applicable to a higher number of chemicals and provide information on the pathways by which adverse outcomes may be induced. Implementing these approaches may help to identify and understand the mechanisms by which chemicals perturb key biological processes of human disease etiology. It may also lead to a more effective and efficient approach for evaluating the large number of data-poor chemicals. Furthermore, these new methods may also address a number of limitations in the existing regulatory testing approach for pesticides, particularly with respect to data-poor formulants and the lack of post-market surveillance.

6.1 THE ROAD AHEAD: THE EVOLUTION OF IATA FROM SCIENTIFIC CONCEPT TO REGULATORY APPLICATION

This report describes a practical approach that could facilitate the incorporation of new scientific knowledge into the regulatory toxicity testing paradigm in a way that would significantly enhance the protection of human health and the environment. It offers a short-term perspective, focusing on strategies that might be realistically adopted over the next decade. Ongoing implementation would facilitate the transition away from an endpoint-driven approach to one anchored in a mechanistic understanding of physiology. In this context, Integrated Approaches to Testing and Assessment (IATA) represent a bridging paradigm that would integrate new science into the existing regulatory framework to enhance the reliability of the existing approach while making it possible to assess the safety of the data-poor chemicals that have not yet received extensive analysis. The Panel's vision for the evolution of IATA in the regulatory context is illustrated in Figure 6.1 and summarized in the following subsections.

6.1.1 Building the Necessary Foundation

Although there is no complete set of alternative methods that can replace the entire testing paradigm for data-rich chemicals today, the science is evolving rapidly. IATA offers a framework by which these advances may be integrated into the regulatory environment in order to augment the existing approach to testing in a transparent and scientifically robust fashion. Adopting IATA benefits all stakeholders — including the regulated community, regulators, and the public — and as a result the Panel believes that the acceptability and applicability of alternative tools will be enhanced by the functional engagement of these communities. This functional collaboration will require active participation of all participants and will necessitate building a foundation whose interdisciplinary nature reflects the diverse nature of toxicology in general.



(Council of Canadian Academies)

Figure 6.1

The Panel's vision for the evolution of IATA in the regulatory context

The adoption of a common vocabulary will be critical to ensuring that all parties can communicate effectively. This vocabulary must be able to integrate terminology from a variety of disciplines. It must also be sufficiently adaptive and responsive to issues that arise from ongoing, international research efforts. Although precedent has been set in other (related) disciplines, there is currently no international initiative to define a standard vocabulary for toxicology that would meet the needs of all stakeholder groups.

As discussed in Chapter 3, the digitization of legacy toxicity data is extremely important for the development of robust databases that could improve prioritization and screening as part of an IATA process. These databases should be built using internationally agreed-upon data standards, ontologies, and software platforms that would permit relational exploration and data mining of all types of toxicity information (e.g., legacy data, new data, bioinformatics data, chemoinformatics data, etc.).

The internationally harmonized nature of pesticide regulations means that any changes to the toxicity testing paradigm would require coordinated global efforts and acceptance across and between national regulatory authorities. As discussed in Chapter 2, there are a several organizations involved in international harmonization of regulatory requirements. Some of these are responsible for developing regulatory policies; others exist to inform policy development but are not themselves regulatory in nature (Table 2.3). The work of these organizations is expected to be instrumental in the evolution of any regulatory reform.

Risk communication is, by its very definition, a dynamic exchange of information between all stakeholders. Sincere and early engagement of all stakeholder groups, coupled with transparency in the regulatory process, will therefore be instrumental in establishing public confidence and trust. As discussed in Chapter 5, the public acceptance of any changes to the regulatory approach to testing and assessment will depend on how these changes are implemented and how the information is communicated to all stakeholders. The concerns of most stakeholders will likely focus on IATA's ability to strengthen the regulatory system, rather than to weaken it in the interests of administrative or economic efficiency. The Panel's review of the evidence suggests that the transition to an integrated approach will significantly augment the existing regulatory framework; therefore, this transition should clearly address the needs and concerns of all stakeholder groups. There is a common belief that misperceptions regarding risk can be solved by simply educating the public about the relevant science. While there is undoubtedly a need to be open and transparent about the scientific basis for any risk-based decision, the relationship between the provision of information and its acceptance is far

from simple. As a result, merely explaining the relevant science will not necessarily lead to higher levels of comfort or acceptance. Different stakeholder groups have different concerns, and therefore different risk communication needs. Appealing to each group on the basis of science alone will not suffice because of the different underlying assumptions and values. Failure by the scientific regulatory community to acknowledge the legitimacy of these concerns — and adequately address them — could significantly erode public confidence and trust. Furthermore, it may lead to the development of an information gap, which would likely be filled by information from alternate sources that may be based on scientifically questionable evidence. Although the concerns and needs of individual stakeholder groups may differ substantially, there are common goals and shared values that can be highlighted in any communication regarding changes to the testing requirements. These include the importance of using the best science to inform decision-making in order to protect human health and promote environmental stewardship.

6.1.2 Evolving the Science Base

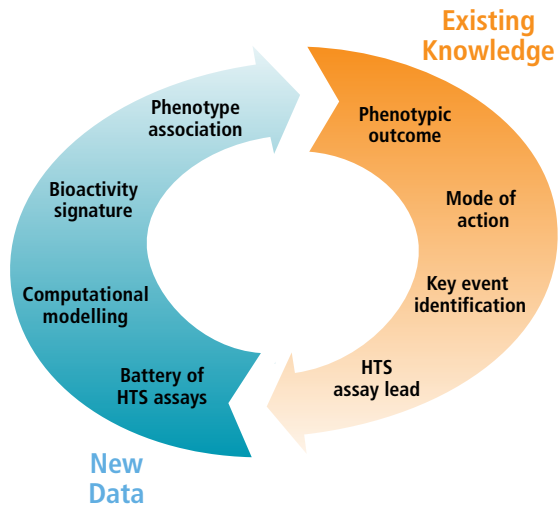
Systems biology lies at the centre of a paradigm shift in regulatory toxicology that could see the field move away from prescribed batteries of apical endpoint tests and towards testing based on a mechanistic understanding of physiology. The interdisciplinary nature of this new approach necessitates functional collaborations between scientists from many disciplines, regulators, and the regulated community. These collaborations will help to ensure that the necessary information and tools are developed appropriately for the intended purpose.

The development of toxicity tests to evaluate perturbation of cellular toxicity pathways is based on a comprehensive understanding of these cellular pathways. In the short term, considerable work will be needed to elucidate the network of biological signalling pathways, to determine what their physiological normal state is, and to distinguish between an adaptive and an adverse response. These pathways would act as examples to illustrate how mechanistically based tests might be developed, validated, and used to inform regulatory decision-making.

The use of high-throughput screening (HTS) assays to generate toxicity data on thousands of industrial chemicals (both data-poor and data-rich) is generating a huge amount of primary *in vitro* data. The rate of data generation is expected to increase over the next 5 to 10 years. The ongoing analyses of these data will be instrumental in identifying emerging patterns and signatures of toxicity in order to elucidate modes of action (MoAs) and build scientifically defensible adverse outcome pathways (AOPs) that can causally relate key events at different levels of biological organization to the *in vivo* endpoint of regulatory interest.

The elucidation of AOPs is fundamental to using an IATA approach and may be achieved by two complementary research approaches (Figure 6.2). The first of these approaches starts at the endpoint of interest and works to identify the perturbed biological pathways; the second starts with pathway assay data and relates patterns of response to disease outcomes. The former is hypothesis-based, exemplified by the AOP approach; the latter is hypothesis-free, exemplified by HTS and computational approaches. The AOP approach may be used to identify key pathways for which HTS approaches could be identified, and the HTS approach may identify key pathways that can be verified using the AOP approach. Together, these synergistic approaches promise improved toxicological understanding, which could facilitate the streamlining of testing and assessment for all chemicals.

Although this report focuses on filling information gaps for data-poor compounds, understanding AOPs or MoAs can also benefit risk assessment for data-rich compounds (e.g., pesticide active ingredients). Once an AOP has been established, the key events can be used for read-across to other chemicals, both qualitatively and quantitatively. If a new compound triggers the key events in an AOP (as determined in a "validated" system), it could trigger the adverse effect of consequence to the MoA. The likelihood will then simply depend on potency (i.e., the dose response for the key events).



(Council of Canadian Academies)

Figure 6.2

AOP/MoA and HTS approaches offer complementary and synergistic approaches to improve toxicological understanding and streamline regulatory testing

The traditional approach to understanding the mode of toxic action for a chemical typically starts with a well-described toxicity of interest (e.g., tumours, malformations, etc). Involvement of the key events in an AOP (or MoA) are established on the basis of the weight-of-evidence (as described by the International Programme for Chemical Safety (IPCS)) using, for example, criteria based on those described by Bradford Hill, which take into account factors such as dose-response and temporal concordance, biological plausibility, coherence and consistency (Hill, 1965). If the causal and dose-response relationships between a key event and adverse outcome are understood, the dose-response for the key event itself can be used as the basis for health-based standards. Understanding the toxicology across a range of MoAs leading to adverse outcomes is required to establish the knowledgebase for eventual streamlining of the testing and assessment of pesticide chemicals. The tiered integration of existing data and *in silico* and *in vitro* models of AOPs will help to focus subsequent testing on those chemicals and endpoints of concern.

Thus, starting with predictive *in vitro* methods to build AOPs for groups or classes of chemicals would be much more efficient and effective than starting with a toxicological outcome for a single chemical. This type of approach could lead to a more efficient testing strategy; every single chemical or endpoint does not need to be evaluated using the same standardized battery of tests. This knowledgebase will take time to build, but as AOPs are established they should be used to make assessment more accurate and efficient.[143]

6.1.3 Evolving the Data Sources and Tools

Computational toxicology permits categorizing chemicals based on their intrinsic properties as a way to screen and prioritize them for further toxicity testing. This is done using relational databases that cross-reference existing data from a multitude of sources. Computational toxicology also harnesses the advances made in systems biology to develop models of predictive toxicity that are anchored in a mechanistic understanding of human physiology.

The Panel believes that, over the next decade, these computational tools will contribute significantly to the elucidation of AOPs and the development of quantitative dose-response prediction models. The tools may also be capable of extrapolating predicted toxicity responses across levels of complexity via the development of multi-stage (virtual tissue) models. This, in turn, may facilitate the evolution of reliable physiologically based pharmacokinetic (PBPK) models.

143 Under the current approach, even active ingredients that are not expected to be toxic are evaluated using the full battery of toxicity tests. As a result, a new herbicide with a known plant-specific pesticidal activity is still subject to as stringent a testing battery as a putative neurotoxin.

These PBPK models could predict the environmental exposure levels below which the resultant tissue concentrations would not trigger an adverse cellular response. The computational tools are expected to increase in complexity and sophistication to the point where, more than 10 years from now, they could be used to model cellular responses to exposure for currently uncharacterized (or unidentified) cellular response pathways.

Proof-of-concept studies using well-characterized AOPs and toxicity endpoints could be instrumental in developing fit-for-purpose tests and facilitating their integration into the existing regulatory system.

Although both toxicity tests and toxicity screens can make use of the same fundamental scientific evidence, they are distinct entities that are used for different (albeit related) purposes. A screen is used to facilitate the rapid analysis of a large number of substances to identify any that may possess characteristics that warrant further investigation. A test is used to generate precise data on specific substances of concern in order to determine their underlying toxicological properties and elucidate dose-response relationships. The Panel anticipates that the use of *in vitro* and *in silico* approaches as components in HTS batteries is a necessary precursor to the evolution of alternative toxicity tests that might be suitable replacements for existing *in vivo* studies in the future.

There are alternative approaches that can replace *in vivo* tests for evaluating acute toxicity endpoints. In the short term, the Panel anticipates that approaches to evaluate critical local effects will also be accepted for regulatory use; however, the development of alternative tests to evaluate more complex toxicity endpoints (e.g., carcinogenicity or reproductive toxicity) will take considerably more time. In the meantime, the Panel anticipates that existing HTS assays may be used to facilitate the screening and prioritization of data-poor industrial chemicals for which decisions are currently made based on little (or no) primary toxicity data. The use of HTS assays to rapidly generate primary toxicity data will be instrumental in demonstrating the utility of these approaches in a regulatory context. Furthermore, the data generated by these assays will also significantly improve the depth of available toxicity data. Analyses of these data may help identify emerging patterns and signatures of toxicity that could be instrumental in helping to further evolve both the fundamental science and testing tools.

6.1.4 A New Role for Population Health

Regardless of the testing methods used to predict toxicological outcomes, laboratory studies will never be completely infallible because they are *in simulacra* (a study

conducted in a model system). They are models that use educated assumptions to represent physiological processes and outcomes in a logical and objective fashion. As a result, post-market surveillance evaluates the conclusions of pre-market laboratory testing. It also identifies those low-incidence effects in target human populations that pre-market testing cannot reliably predict. In this regard, it represents the truest form of validation for any laboratory study, regardless of the model used (i.e., *in vivo*, *in vitro*, or *in silico*).

The limited availability of scientifically robust population-level studies that can reliably establish (or refute) causal linkages between exposure and adverse health outcomes represents an important limitation in the current regulatory system for pesticides. The current system relies heavily on pre-market toxicity tests and cannot readily accommodate the inclusion of epidemiological data. This is partly because a product in the pre-market phase of regulation has never been used in the environment and thus population-level data would be unavailable. Nonetheless, the Panel believes that IATA principles, combined with advances in computational toxicology and systems biology, could facilitate the inclusion of epidemiological data in the pre-market evaluation of pesticide active ingredients. This would increase the reliability of predicted risk (Figure 3.17). Indeed, population surveillance studies that collect quantitative exposure data in an orderly and systematic fashion are necessary to establish (or refute) causal relationships between exposure and adverse health outcomes at the population level. They are also prerequisites to the evolution and validation of reliable PBPK models that can relate *in vitro* assay data and *in vivo* tissue doses to population level exposures and adverse health outcomes.

Precedent for post-market surveillance of regulated chemicals has been set in the pharmaceutical industry where population monitoring constitutes an important component of the regulatory process. Despite the inherent differences between pharmaceutical drugs and environmental chemical exposure, the Panel believes that post-market surveillance studies to capture pesticide exposure are necessary to improve the current understanding of exposure in human populations and the health effects of regulated agents. Furthermore, where population-level epidemiological data are available for a given chemical, their consideration in the pre-market risk assessments of related chemicals would represent an opportunity to increase the reliability of predictions.

Besides identifying any unanticipated effects of pesticide exposure, there is a need for a mechanism that would collect exposure information in an orderly and disciplined way so as to follow up on reported or suspected adverse effects of pesticide exposure. The existence of such a system is a prerequisite to including

epidemiological data in relational databases that would permit its consideration in pre-market risk assessments.

Although population-level studies identifying adverse effects of exposure to environmental chemicals are inherently challenging (see Section 2.5.4), there are a number of well-designed, ongoing epidemiological studies that are applicable to environmental exposures and that represent excellent models for the collection of these kinds of data. The U.S. Agricultural Health Study (AHS) was started in 1993 by researchers from the U.S. National Cancer Institute, the NIEHS, and the US EPA. In 2011 it had enrolled almost 90,000 participants.[144] It has proven to be a productive platform from which studies of both cancer and non-cancer outcomes, biomarkers, and exposure have been conducted. Despite the geographic challenges of conducting studies in a country as large as Canada, recent biomonitoring initiatives by Health Canada show the feasibility of large-scale, scientifically rigorous epidemiological studies of the geographically dispersed Canadian population (Health Canada, 2010b).

The Panel anticipates that advances in systems biology will help develop and apply biomarkers of exposure and intermediate metabolites and permit consideration of genetic susceptibility in epidemiological studies of environmental risk factors. In the short term, identifying specific biomarkers of pesticide exposure might permit the collection of pesticide-related data as part of existing population surveillance initiatives. In the long-term, the inclusion of exposure data from post-market surveillance studies of structurally related chemicals in relational databases would permit the consideration of epidemiological data in pre-market risk assessments. These advances could facilitate the meaningful integration of quantitative epidemiological data into the regulatory risk assessment process in a pragmatic but revolutionary way.

6.1.5 Evolving the Regulatory Process

The current risk assessment processes are predicated on the types of data that have historically been generated by toxicity testing. The nature of the data generated by alternative testing methods may not be of use in the current regulatory framework. As a result, the Panel expects that the nature of an IATA strategy will vary depending on the type of chemicals in question and the nature of the decision-making process that the data are intended to inform.

For data-poor chemicals, the lack of data supporting rational hypotheses for a plausible toxicological potential may be the impetus for a new approach. Data-rich

144 Agricultural Health Study: http://aghealth.nci.nih.gov/

chemicals are already subject to an extensive battery of toxicity tests; therefore establishing relevance may take longer and will be predicated on building and establishing trust in new and novel methods. Although adopting IATA strategies might refine and streamline the testing for these chemicals as well as enhance the reliability of the outcome, the Panel does not anticipate a widespread deployment of IATA in the short term.

The dynamic nature of IATA necessitates a new approach to test development, validation, and regulatory acceptance. Test development should be based on a functional collaboration between regulators and scientists to ensure that tests evolve to fit the needs of the testing paradigm. This should be coupled with capacity-building initiatives within the regulatory community to develop comfort with the science underpinning the alternative tests, and to build familiarity with the data these tests produce.

Alternative tests should be evaluated using performance-based standards that judge the utility of a test against knowledge of the underlying biology. These test methods typically target specific cellular or physiological responses and, as such, preclude validation with *in vivo* data by a one-for-one approach. The AOP allows for the use of a suite of models or assays that are designed to target particular steps along a specific pathway. The scientific justification of an alternative method or data set should therefore focus on comparing the test outcome to what is known about the underlying biology as described in the AOP. In turn, the scientific validation of an alternative test method would be based on mechanistic endpoints that would be measured in assays designed to evaluate a specific cellular or physiological response.

6.2 EVOLVING IATA: INTEGRATING SCIENCE AND REGULATION

Although the existing approach to toxicity testing for data-rich chemicals is well established, opportunities now exist for incorporating new scientific approaches that may improve hazard assessment. These new approaches include high-throughput technologies, both experimental and computational, that provide an opportunity to generate mechanism-centred data on a much larger number of chemicals and biological pathways. These tools may help in prioritizing chemicals, which could allocate greater resources to those substances of most concern. In addition, these tools may help to transition regulatory toxicology away from a focus on *what* happens and towards an understanding of *how* it happens.

The utility of IATA is rooted in elucidating biological mechanisms that explain toxicological effects. IATA necessitates a dynamic approach that will ensure a continued evolution to expand its applicability to the regulatory context as the state of science continues to advance. For this reason, it is impossible to predict precisely what the long-term vision of an IATA approach to regulatory toxicology may be. Suffice it to say that it necessitates a more agile, responsive, and mechanism-based testing approach that can exploit state-of-the-art techniques. Any regulatory changes will face a number of scientific and policy challenges, but these will also come with a number of opportunities. The successful implementation of IATA will require a concerted effort by — and sustained dialogue between — all stakeholder groups including scientists, regulators, policy-makers, and the public.

The state of the science is evolving rapidly, and opportunities exist to address some of the limitations that could not be addressed until now. Furthermore, continued advances will likely identify new limitations. These 21st century problems need 21st century solutions. Although IATA may not be able to address all of these issues, it represents a transparent and pragmatic blueprint for change.

6.3 CHAPTER SUMMARY

What is the scientific status of the use of integrated testing strategies in the human and environmental regulatory risk assessment of pesticides?

To date, aspects of computational toxicology (i.e., the use of alternative approaches to traditional animal testing) have primarily been used to support regulatory decision-making for data-poor chemicals such as pesticide formulants. Although the Panel is not aware of a complete set of alternative methods that could replace the entire testing paradigm today (even for data-poor chemicals), the state of the science is evolving rapidly. With the continued development of such tools and approaches, the Panel expects to see the increased use of integrated testing strategies in decision-making, with an eventual adaptation to inform decisions involving data-rich chemicals. As such, these emerging technologies, integrated with existing data, are a pragmatic means by which new testing methods could be used to augment the regulatory paradigm and help bridge the transition to a hypothesis-driven approach to testing and assessment.

Appendices

Appendix A: Technical Glossary*

Acceptable Daily Intake (ADI)

Based on available toxicity data, the amount of a chemical that can be ingested (orally) in food or drinking water, on a daily basis over a lifetime, without an appreciable risk to human health. Typically expressed in milligram of chemical per kilogram bodyweight (mg/kg).

Active Ingredient

The component within a pest control product to which the intended effects may be attributed. This is the ingredient that controls the pest, and it must be clearly identified on the product label.

Acute Exposure

Exposure to a substance for a short period of time. In toxicology this is defined as fewer than 14 days; for pesticide exposure a period of 24 hours is generally used (compare: Intermediate Duration-Exposure and Chronic Exposure).

Acute Reference Dose (ARfD)

The maximum dose to which an individual could be exposed in a day with no expected adverse health outcomes. The US EPA equivalent is the Acute Population Adjusted Dose (aPAD).

Adaptive Response

Changes that occur (typically in response to exposure) that permit a return to the normal (homeostatic) state without any irreversible disruptions to the overall system.

Adverse Outcome Pathway (AOP)

The sequence of events from the chemical structure through the molecular initiating event to the *in vivo* outcome of interest.

Adverse Response

Changes that occur that result in impairment of functional capacity, often due to an insult that exceeds the capacity of the adaptive response to permit a return to the homeostatic state. Outcomes might include changes in morphology, development, lifespan, or growth of the organism. At the molecular level, responses might include alterations in gene expression, protein synthesis, or cell cycle regulation.

* Key terms as used by the Panel throughout this report.

Animal Model
A laboratory animal used as a human surrogate in order to identify potential adverse health outcomes due to toxicant exposure.

Animal Testing
The use of non-human animals in experiments, typically as surrogates for human exposure to a substance.

Apical Endpoint
An observable outcome from an animal test that is used as an indicator of toxicity — for example, growth defects, developmental issues, tumour formation, mortality, or disease progression. Apical endpoint tests evaluate the end result of exposure but provide little or no information about the mechanism by which the response occurred.

Applicability Domain
The physicochemical, structural, or biological space and information that was used to develop a (Q)SAR model, and for which that model gives predictions with a given level of reliability (Netzeva *et al.*, 2005).

Assay (Bioassay)
A form of scientific experiment. The experimental process for determining the effects of a test substance on a biological system.

Benchmark
A standard against which something can be judged. In the case of regulatory toxicology, the existing safety standards provide the benchmark against which new tests will be judged.

Benchmark Dose (BMD)
The dose projected (from a fitted mathematical model) to cause a prespecified level of change from the control in an exposure response. BMDs typically serve as the points of departure to assess the potential risks posed by the various exposure scenarios.

Bioavailability
The fraction of the external dose that reaches the systemic circulation of an organism. Bioavailability differs depending on route of exposure (e.g., intravenous administration is assumed to result in complete bioavailability). Bioavailability declines when exposure is mediated via other routes (e.g., oral, topical, etc.).

Biochemical Pathway
A series of reactions, typically enzyme-catalyzed, that are associated with a specific physiological event in a living organism.

Biochemistry
The study of the chemical processes and substances that occur in living organisms.

Bioinformatics
Applying the tools of information technology to biology (compare: Computational Toxicology).

Carcinogenicity
The degree to which an agent is capable of inducing malignant neoplasms.

Cell Line
Cells (human, animal, or plant) of a single type that have been adapted to grow continuously in the laboratory and are used in research.

Chiral
A chemical or molecule that lacks an internal plane of symmetry (compare: Enantiomers).

Chemoinformatics
The application of information technology to the field of chemistry. Also known as computational chemistry. Analogous to bioinformatics.

Chronic Exposure
Exposure to a substance for a period of time that is greater than one year (compare: Acute Exposure and Intermediate-Duration Exposure).

Chronic Reference Dose
The maximum dose to which an individual could be exposed over a lifetime with no expected adverse health outcomes. The US EPA equivalent is the Chronic Population Adjusted Dose (cPAD).

Computational Toxicology
The use of mathematical and computer models to predict adverse effects and to better understand the mechanisms by which a particular substance elicits an effect. Bioinformatics is a discipline in this field (compare: Bioinformatics).

Concentration
The quantity of a chemical substance contained in a unit amount of another substance (compare: Dose).

Cytotoxicity
The degree to which an agent causes damage to cell structure or function.

Database (DB)
A digital system that is organized to permit the rapid search, retrieval, modification, and deletion of data.

Dose
The quantity of a chemical substance to which an organism is exposed (compare: Concentration).

Dose-Response Relationship
The relationship between the amount of a substance to which an organism is exposed (i.e., the dose) and the magnitude of the observed response.

Dread Risk
A risk of harm or adverse effects that invoke particularly high levels of negative emotion, fear, or even terror.

Ecotoxicology
The study of the toxicology applied to all living organisms, including the effects on ecosystems, communities, and populations.

Enantiomers
A pair of stereoisomers that are non-superimposable mirror images of each other (compare: Chiral).

Endocrine Disruptor
An exogenous substance that can change endocrine function and cause (potentially adverse) effects at the level of the organism, its progeny, and/or (sub)populations of organisms.

Epigenetics
Changes in an organism caused by mechanisms other than changes in the DNA sequence. These changes may persist through cell division, and may even be passed to subsequent generations, but there is no change in the underlying DNA sequence of the organism.

Exposure
Contact with a substance by ingestion, inhalation, or contact with the skin or eyes. Exposure may be short term (acute), of intermediate duration, or long term (chronic). The magnitude of exposure to a particular agent that reaches the target population, organism, organ, tissue, or cell is usually expressed as a number that defines concentration, duration, frequency, or intensity.

Exposure Pathway
The analysis of the route a substance takes from its source to its endpoint, and the means by which individuals were exposed to it.

An exposure pathway has five parts: a source of contamination (such as an abandoned mine); an environmental medium and transport mechanism (such as movement through groundwater); a point of exposure (such as a private well); a route of exposure (eating, drinking, breathing, or touching); and a receptor population (people potentially or actually exposed).

False Negative
Also know as a Type II error. An experimental result that is erroneously negative.

False Positive
Also known as a Type I error. An experimental result that is erroneously positive.

Formulant
A non-active ingredient added to a pest control product, typically to improve its properties. Also known as inert.

Genetic Polymorphism
Inter-individual differences in the sequence of a specific gene. This phenomenon often gives rise to different appearances and traits in the organism.

Genome
The full DNA sequence of an organism.

Genomics
The study of genes and their functions. Genomics examines the interplay among molecular mechanisms, genetic factors, and environmental influences.

Genotoxicity
The degree to which an agent causes damage to genetic material.

Genotype
The genetic constitution of an organism with respect to the characteristic under consideration, i.e., the specific allelic complement of an individual that determines a specific trait (compare: Phenotype).

Good Laboratory Practice (GLP)
As defined by the OECD, GLP is a "quality system concerned with the organisational processing process and conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived, and reported" (OECD, 2004f). GLP controls are designed and enforced to ensure results are consistent, reliable, and reproducible.

Hazard
The inherent toxicity of the chemical of interest. This is an intrinsic property of the substance.

High-Throughput Screening (HTS)
An approach that uses automated tools to facilitate the rapid execution of hundreds of thousands of assays per day in order to identify chemicals of concern for subsequent testing.

High-to-Low-Dose Extrapolation Modelling
The process of predicting low exposure risk to humans and animals based on high-exposure, high-risk data obtained from laboratory animals.

Hypothesis-Driven
Approaches to science may be generalized as either descriptive or hypothesis-driven. Hypothesis-driven approaches are those that start by defining the key components that characterize the endpoint(s) of interest. In the context of toxicity testing, a hypothesis-driven approach begins by examining the chemical of interest in order to identify structural characteristics that confer toxicological potential. Subsequent steps narrow the focus to specific toxicity endpoints based on a mechanistic understanding of the interactions between the chemical and biological system.

Informatics
An interdisciplinary field that studies the analysis, collection, classification, digitization, dissemination, manipulation, storage, and retrieval of data. Subdisciplines are concerned with data from biological (bioinformatics) and chemical (chemoinformatics) sources.

Integrated Approaches to Testing and Assessment (IATA)

A tiered approach to data gathering, testing, and assessment that integrates different types of data (including physicochemical and other chemical properties as well as *in vitro* and *in vivo* toxicity data). When combined with estimates of exposure in an appropriate manner, the IATA provides predictions of risk. In an IATA, unsuitable substances are screened out early in the process. This reduces the number of substances that are subjected to the complete suite of regulatory tests. Plausible and testable hypotheses are formulated based on existing information and/or information derived from lower tier testing and only targeted testing is performed in the higher tiers. Failure to satisfy the toxicity requirements at a lower tier typically precludes further testing at a higher tier.

Interactome

All of the interactions between the biological constituents of a system.

Intermediate-Duration Exposure

Exposure to a substance for a period of time that is greater than 14 days but less than 1 year (compare: Acute Exposure and Chronic Exposure).

In chemico

Abiotic measurements of the reactive properties of a chemical agent.

In silico

Performed on a computer or by computer simulation.

In vitro

In an artificial biological environment outside of a living organism.

In vivo

Within a living organism. For example, toxicity tests conducted using animal models.

Knowledgebase (KB)

A type of database that provides a mechanism to collect and organize knowledge in order to facilitate its retrieval in an intelligent and facile manner. Knowledgebases are able to integrate expert knowledge and produce outputs that are capable of handling complex rules, case-based reasoning, probabilistic reasoning, fuzzy logic, and other forms of artificial intelligence.

Macromolecule
A large and complex molecule. In biochemistry, these include nucleic acids (RNA and DNA), proteins, and polysaccharides as well as non-polymeric substances of large molecular mass.

Margin of Exposure (MoE)
Ratio of NOAEL to the theoretical, predicted, or estimated exposure dose or concentration. In more general terms, this is the ratio of the point of departure to the exposure.

Margin of Safety (MoS)
The margin between the reference dose (RfD) and the actual exposure dose or concentration.

Metabolism
The conversion or breakdown of a substance from one form to another by a living organism. This includes the uptake and distribution, within the body, of chemical compounds; the changes (biotransformation) undergone by such substances; and the elimination of the compounds and of their metabolites.

Metabolite
Any intermediate product or end product resulting from metabolism.

Mitochondriomics
The application of "omics" (e.g., genomics, proteomics, and transcriptomics) and bioinformatics to the study of mitochondria.

Mode of Action (MoA)
The sequence of key cellular and biochemical events (measurable parameters), starting with the interaction of an agent with the target cell, through functional and anatomical changes, resulting in cancer or other adverse health effects. Mode of action differs from mechanism of action in that the latter describes the complete molecular sequence of events from exposure to manifestation of the toxicological outcome and implies a more detailed understanding of causality leading to an adverse outcome (Seed *et al.*, 2005).

Molarity
The concentration of a substance in solution, expressed as the number of moles of solute per litre of solution.

Mutation
A change in the nucleotide sequence of an organism's genetic material.

Neurotoxicity
The ability of a substance to cause adverse effects on the nervous system.

No Observed Adverse Effect Level (NOAEL)
An exposure level at which there is no statistically or biologically significant increase in adverse effects in the exposed population as compared to the appropriate control.

Omics
A term used to encompass fields of biological study that end in –omics. These include genomics, proteomics, metabolomics, and toxicogenomics. In molecular biology, the suffix –*ome* is typically used to describe fields that endeavour to consider constituent components collectively as part of a larger system. For example, the application of genomics technologies (including HTS assays) to the field of toxicology is termed toxicogenomics.

Ontology
A term which refers to a controlled vocabulary for describing gene product characteristics such as a cellular compartment, molecular function, and biological processes.

Perturbation
A change in the biological system in response to exposure to a given substance.

Pest
Any injurious, noxious, or troublesome insect, fungus, bacterial organism, virus, weed, rodent, or other plant or animal.

Pest control Product (PCP)
Any product, device, organism, substance, or thing that is manufactured, represented, distributed, or used to control, prevent, destroy, mitigate, attract, or repel a pest (Government of Canada, 2002a). All PCPs sold in Canada must have a product label that includes specific information regarding the active ingredients, the formulation, the intended use of the product, and the identity of the registrant. This label is a legal document that follows a standardized format.

Pesticide
The end-use pest control product. The pesticide typically contains a mixture of active ingredient and formulants.

Pharmacokinetics
The study of the process by which a substance is absorbed, distributed, metabolized, and excreted by a biological system. Pharmacokinetics can be used to establish quantitative relationships between dose, concentration, and time.

Pharmacology
The study of drugs, including the body's reaction to them.

Phenotype
The observable characteristics of an organism (colour, size, etc.) that result from the interaction of the organism's total genetic makeup, or genotype, with the environment (compare: Genotype).

Physicochemical Properties
The physical and chemical characteristics of a substance.

Physiologically Based Pharmacokinetic (PBPK) Modelling
PBPK models are generally multi-compartment mathematically designed to predict the absorption, distribution, metabolism, and excretion (ADME) of substances by an organism. In a typical PBPK model, individual compartments correspond to different organ systems. PBPK models are often used to conduct interspecies extrapolations and to generate simulations of pharmacokinetic profiles under different physiological conditions.

Predictive Validity
Reliability of a measurement expressed in terms of its ability to predict the criterion.

Predictivity
The prognostic power of a test as defined by its relevance and reliability to predict an outcome in humans.

(Quantitative) Structure-Activity Relationship ((Q)SAR)
A mathematical relationship that (quantitatively) links chemical structure and physicochemical properties to a well-defined process, such as biological activity or reactivity.

Reference Dose (RfD)
Term used to estimate maximum daily exposure to a substance by the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime.

Refine/Reduce/Replace ("The Three Rs")

In 1959, Russell and Burch proposed that scientists refine experimental procedures to eliminate causes of pain and distress to laboratory animals; reduce the number of animals to the minimum required to achieve the experimental end; and replace animals whenever possible with either cell and tissue culture models (*in vitro* studies) or other methodologies.

Reverse Pharmacokinetics

An approach that extrapolates from an effective *in vitro* concentration to an equivalent human exposure level (or dose).

Risk

The likelihood that a subject will be harmed, or experience an adverse health outcome, if exposed to a particular hazard. Risk is a function of both the probability of exposure and the intrinsic hazard of the substance.

Risk Communication

A reciprocal process based on an interactive dialogue between all stakeholders affected by a particular risk.

Risk Perception

A subjective judgment regarding the characteristics, severity, and acceptablility of a risk.

Screening

Tests used to identify undetected abnormalities, unrecognized diseases, or defects. Pharmacological or toxicological screening typically uses a standardized set of procedures that examine a range of compounds to determine their pharmacological and toxicological properties and to establish a dose-response and dose-effect relationship.

Stakeholder

An individual, group, or organization that affects or may be affected by an organization's actions.

Systems Biology

A field of study that seeks to identify and understand the implications of molecular and signalling interactions that take place within cells, and how these interactions result in the functions and behaviours exhibited by biological systems.

Teratogenicity
The potential of an agent to induce structural deformations in the offspring when administered prenatally to the mother.

Threshold of Toxicological Concern (TTC)
The maximum level of human intake or exposure that is considered to be of negligible risk. Provided that a TTC value can be derived, the TTC can be used as a surrogate for safety data in the absence of chemical-specific primary toxicity data.

Toxicity
The ability of a substance to cause injury or adverse effects with reference to the quantity administered or absorbed, the mode of administration, the duration of exposure, the severity of the response, the time needed to produce the response, the nature of the affected organism(s), and other relevant conditions. A measure of the incompatibility of a given substance with life.

Toxicity Pathway
A cellular response pathway that, when sufficiently perturbed, would be expected to result in adverse health effects (NRC, 2007).

Toxicity Screen
An experimental approach designed to facilitate the rapid analysis of a large number of chemicals in order to identify any that may warrant further (more specific) investigation. Screens would typically be used early in a tiered approach. Screens are generally high-throughput and highly sensitive. In toxicity screens, false positives are tolerated but false negatives are not.

Toxicity Test
An experimental approach designed to generate specific toxicity data on a chemical in order to characterize its intrinsic toxicological properties.

Transcriptomics
The study of genome-wide mRNA expression profiles.

Validation
The process of testing the reliability and relevance of a test method. Reliability considers the reproducibility of test results. Relevance describes the usefulness of the data produced for their intended purpose.

Appendix B

Test Requirements

Study Type	Name of Test	Preferred Model System	PMRA Data Code (DACO)	US EPA Test Guidelines	OECD Test Guidelines	References
4.2 Acute studies – TGAI	Acute oral	Rat	4.2.1	OPPTS 870.1100	OECD 420	(OECD, 2001a, 2001b, 2008a; US EPA, 2002a)
					OECD 423	
					OECD 425	
	Acute dermal	Rat, rabbit, or guinea pig	4.2.2	OPPTS 870.1200	OECD 402	(OECD, 1987; US EPA, 1996)
	Acute inhalation	Rat	4.2.3	OPPTS 870.1300	OECD 403	(OECD, 2004b, 2004d; US EPA, 1998b)
					OECD 433 (draft)	
	Primary eye irritation	Albino rabbit	4.2.4	OPPTS 870.2400	OECD 405	(OECD, 2002c; US EPA, 1998c)
	Primary dermal irritation	Albino rabbit	4.2.5	OPPTS 870.2500	OECD 404	(OECD, 2002b; US EPA, 1998d)
	Dermal sensitization	Mouse	4.2.6	OPPTS 870.2600	OECD 406	(OECD, 1992, 2002d; US EPA, 2003b)
					OECD 429	
	Potentiation/Interaction	n/a	4.2.7	n/a	n/a	
	Antidote	n/a	4.2.8	n/a	n/a	
	Other acute studies	n/a	4.2.9	n/a	n/a	
4.3 Short-term oral studies – TGAI	Short-term oral (90-day rodent)	Rat	4.3.1	OPPTS 870.3100	OECD 408	(OECD, 1998b; US EPA, 1998e)
	Short-term oral (90-day and/or 12-month dog)	Dog	4.3.2	OPPTS 870.3150	OECD 409	(OECD, 1998a; US EPA, 1998f)
		Rat		OPPTS 870.4100	OECD 452	(OECD, 2009e; US EPA, 1998l)
	Short-term oral (28-day)	Rat	4.3.3	OPPTS 870.3050	OECD 407	(OECD, 2008b; US EPA, 2000)

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Appendix B (continued)

Test Requirements

Study Type	Name of Test	Preferred Model System	PMRA Data Code (DACO)	US EPA Test Guidelines	OECD Test Guidelines	References
4.3 Short-term oral studies – TGAI *(continued)*	Short-term dermal	Rat, rabbit, or guinea pig	4.3.4	OPPTS 870.3250	OECD 411	(OECD, 1981b; US EPA, 1998h)
	Short-term dermal (21/28-day)	Rat, rabbit, or guinea pig	4.3.5	OPPTS 870.3200	OECD 410	(OECD, 1981a; US EPA, 1998g)
	Short-term inhalation (90-day)	Rat	4.3.6	OPPTS 870.3465	OECD 413	(OECD, 2009h; US EPA, 1998i)
	Short-term inhalation (21/28-day)	n/a	4.3.7	n/a	OECD 412	(OECD, 2009g)
	Other short-term studies	n/a	4.3.8	n/a	n/a	
4.4 Long-term studies – TGAI	Chronic (rodent)	Rat	4.4.1	OPPTS 870.4100	OECD 452	(OECD, 2009e; US EPA, 1998l)
	Oncogenicity (rodent species 1)	Rat, mouse	4.4.2	OPPTS 870.4200	OECD 451	(OECD, 2009d; US EPA, 1998m)
	Oncogenicity (rodent species 2)		4.4.3			
	Combined chronic/Oncogenicity (rodent)	Rat	4.4.4	OPPTS 870.4300	OECD 453	(OECD, 2009f; US EPA, 1998n)
	Other long-term studies	n/a	4.4.5	n/a	n/a	
4.5 Special studies – TGAI	Multi-generation reproduction (rodent)	Rat	4.5.1	OPPTS 870.3800	OECD 416	(OECD, 2001d; US EPA, 1998k)
	Prenatal developmental toxicity (rodent)	Rat, rabbit	4.5.2	OPPTS 870.3700	OECD 414	(OECD, 2001c; US EPA, 1998j)
	Prenatal developmental toxicity (non-rodent)		4.5.3			
	Genotoxicity: bacterial reverse mutation assay	At least five strains of bacteria *(Salmonella typhimurium)*	4.5.4	OPPTS 870.5100	OECD 471	(OECD, 1997a; US EPA, 1998o)

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Appendix B (continued)

Test Requirements

Study Type	Name of Test	Preferred Model System	PMRA Data Code (DACO)	US EPA Test Guidelines	OECD Test Guidelines	References
4.5 Special studies – TGAI *(continued)*	Genotoxicity: *in vitro* mammalian cell assay	Cell types used in this test should have a demonstrated sensitivity to chemical mutagens, a high cloning efficiency and a stable spontaneous mutant frequency.	4.5.5	OPPTS 870.5300	OECD 476	(OECD, 1997b; US EPA, 1998u)
	Genotoxicity: *in vitro* mammalian clastogenicity	A variety of cell lines, strains or primary cell cultures, including human cells, may be used (e.g., Chinese hamster fibroblasts, human or other mammalian peripheral blood lymphocytes).	4.5.6	OPPTS 870.5375	OECD 473	(OECD, 1997c; US EPA, 1998v)
		A variety of cell lines, strains or primary cell cultures, including human cells.		OPPTS 870.5900	OECD 479	(OECD, 1986d; US EPA, 1998dd)
	Genotoxicity: *in vivo* cytogenetics	Rat, mouse	4.5.7	OPPTS 870.5395	OECD 474	(OECD, 1997e; US EPA, 1998y)
		Rat, mouse, chinese hamster		OPPTS 870.5385	OECD 475	(OECD, 1997d; US EPA, 1998x, 1998ee)
		n/a		OPPTS 870.5915		

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Appendix B (continued)

Test Requirements

Study Type	Name of Test	Preferred Model System	PMRA Data Code (DACO)	US EPA Test Guidelines	OECD Test Guidelines	References
4.5 Special studies – TGAI *(continued)*	Other genotoxicity studies	Ascomycete fungus *(Apergillus nidulans)*	4.5.8	OPPTS 870.5140	OECD 477	(OECD, 1984b; US EPA, 1998p)
		Mouse		OPPTS 870.5195	OECD 478	(OECD, 1984a; US EPA, 1998q)
		Mouse		OPPTS 870.5200	OECD 480	(OECD, 1986b; US EPA, 1998r)
		Ascomycete fungus *(Neurospora crassa)*		OPPTS 870.5250	OECD 481	(OECD, 1986f; US EPA, 1998s)
		Fruit fly *(Drosophila melanogaster)*		OPPTS 870.5275	OECD 482	(OECD, 1986c; US EPA, 1998t)
		Chinese hamster		OPPTS 870.5380	OECD 483	(OECD, 1997f; US EPA, 1998w)
		Rat, mouse		OPPTS 870.5450	OECD 484	(OECD, 1986e; US EPA, 1998z)
		Mouse		OPPTS 870.5460	OECD 485	(OECD, 1986a; US EPA, 1998aa)
		n/a		OPPTS 870.5500	OECD 486	(US EPA, 1998bb, 1998cc)
		A variety of cell lines or primary cell cultures, including human cells, may be used in the assay.		OPPTS 870.5550		
		Yeast *(Saccharomyces cerevisiae)*		OPPTS 870.5575		

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Appendix B (continued)

Test Requirements

Study Type	Name of Test	Preferred Model System	PMRA Data Code (DACO)	US EPA Test Guidelines	OECD Test Guidelines	References
4.5 Special studies – TGAI *(continued)*	Metabolism/Toxicokinetics in mammals (laboratory animals)	Rat	4.5.9	OPPTS 870.7485	OECD 417	(OECD, 1984c; US EPA, 1998hh)
	Delayed neurotoxicity (hen)	Domestic laying hen *(Gallus gallus domesticus)*	4.5.10	OPPTS 870.6100	OECD 418	(OECD, 1995a, 1995b; US EPA, 1998ff)
	28-Day delayed neurotoxicity (hen)		4.5.11		OECD 419	
	Acute neurotoxicity	Rat	4.5.12	OPPTS 870.6200	n/a	(OECD, 1997g; US EPA, 1998gg)
	90-Day neurotoxicity		4.5.13		OECD 424	
	Developmental neurotoxicity	Rat	4.5.14	OPPTS 870.6300	OECD 426	(OECD, 2007e; US EPA, 1998ii)

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