Section 1
Introduction

1

Quantitative Modeling in Toxicology: An Introduction

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1.1 Introduction

1.1.1 Models and Modeling – Definitions

Models are simplified representations of a system with the intent of reproducing or simulating the structure, function or behavior of the system. Depending upon the goal, the models can be physical, conceptual, or mathematical. The mathematical models, also referred to as quantitative models, correspond to one or more equations whose solution provides the time-space evolution of the state variable (Bellomo and Preziosi, 1995). Quantitative modeling is, therefore, the process of developing mathematical descriptions of the interrelationships among input parameters in order to adequately simulate the system behavior (i.e., generate model output).

The quantitative models can be classified in a number of ways. For example:

- Discrete or continuous
- Deterministic or stochastic
- Empirical or mechanistic.

A quantitative model is *discrete* if the state variable does not depend upon the time variable (Bellomo and Preziosi, 1995); the *continuous or dynamic* models describe the change in state variable over time. A model is *deterministic* if its outcome is a direct consequence of

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the initial conditions, not influenced by any random factors; it is *stochastic or probabilistic* when some or all features of the model capture a random behavior. The *empirical or data based* models correspond to equations that emulate the observed data. These models require no prior knowledge of the system but require that both the input and output be known a priori. An example of this kind of models is the one-compartmental pharmacokinetic model, which describes the relationship between the blood concentration at any time t (C_t) as a function of initial concentration (C_o) and elimination rate (k):

$$C_t = C_o \, \mathrm{e}^{-kt}$$

The *mechanistic* models are based on "first principles" or key mechanisms of the process of interest. Here, the compartments mimic system elements and the equations describe the quantitative relationship among system elements or key parameters to generate predictions of system behavior. Many simulation models in practice, however, may consist of mechanistic and empirical components. The motivation for the use of quantitative simulation models in toxicology is related to one or more of the following needs (Andersen, Clewell, and Frederick, 1995):

- Organize and codify facts and beliefs.
- Expose contradictions in existing data/beliefs.
- Explore implications of beliefs about the system.
- Expose serious data gaps.
- Predict response under new conditions.
- Predict parameter values for "inaccessible" parameters.
- Identify essentials of system structure.
- Provide representation of current state of knowledge.
- Suggest and prioritize new research.

In the follow section, a short review of chemical risk assessment is provided to help understand the motivation for developing quantitative systems modeling in toxicology.

1.1.2 Evolution of Chemical Risk Assessment

To a large extent, the development of quantitative modeling tools in toxicology parallels the increasingly sophisticated understanding of modes of action of toxic chemicals in the body and the desire to apply this information to improve quantitative chemical risk assessment. In this regard, mode of action represents the nature of the initial interactions between a toxic compound and the biological system, and the steps that ensue from this interaction leading to adverse downstream consequences for the organism. Initially, little information on modes of action was available. In safety assessments in the 1950s for instance, animal toxicity test results were used to determine No-Observed Effect Levels (NOELs). The US Food and Drug Administration (FDA) derived acceptable daily intakes (ADIs) by dividing animal NOELs by 100 (Lehman and Fitzhugh, 1954). The factor of 100 consisted of two safety factors of 10 each, intended in a general way to account for (1) differences in sensitivity of humans compared to animals and (2) variation in sensitivity of individuals in a heterogeneous human population compared to more homogeneous sensitivity in inbred

animal stains. These ADIs were usually established based on organ or organism level responses that were clearly adverse to health. An underlying premise in this approach was the existence of a threshold dose, that is, the belief that there were concentrations or exposure levels below which the risk of adverse health effects was zero. The dose measure for these evaluations was administered dose, for example, ppm in air or food or mg/kg for orally administered materials.

The 1970s brought a focus on the biology of cancer and a shift of testing and research resources in toxicology toward chemical carcinogenesis (Albert, Train, and Anderson, 1977). Animal studies provided information of the incidence of tumors at specific doses in test animals, usually rats and mice. Two extrapolations were introduced: one predicted the shape of the dose-response curve at low levels of response; the second adjusted the expected responses for different species. The low dose extrapolation used a mathematical model of carcinogenesis, the linearized multistage (LMS) model. This model predicted some probability of increased cancer incidence at every dose, no matter how small. Interspecies extrapolation was calculated on a surface area adjustment for dose. This body surface extrapolation regarded humans as more sensitive to toxic responses than the smaller rodent species.

The concept of dose was initially refined by toxicologists who borrowed methods from the field of clinical pharmacokinetics (PK) to assess the relationship between exposure, sometimes called administered dose, and the concentrations of active chemical/metabolites at target tissues. The initial emphasis on pharmacokinetic modeling in toxicology arose mainly due to the high doses used in many animal tests, doses at which capacity limited processes, that is, metabolism, tubular excretion in kidney, and so on, became saturated. Work with vinyl chloride carcinogenesis showed a better relationship between metabolized dose of this compound and liver cancer rather than a correspondence with inhaled concentration (Watanabe and Gehring, 1976).

The 1980s provided other important developments that would shape the need for quantitative modeling tools. Among them were increasing use of *in vitro* cell systems for assessing chemical interactions in living cells and the first applications of molecular techniques emerging from the new field of molecular biology. Receptor-mediated toxicity, such as dioxin interactions with the Ah receptor, gained prominence. Another advance was the increasing sophistication applied to assessing how chemicals caused their effects – the mode of action of chemicals in biological systems. These contributions provided pressure to apply this growing information in some manner to improve the scientific basis of chemical risk assessment.

1.1.3 Risk Assessment Guidance

A seminal publication (NRC, 1983) proposed a set of consistent "inference guidelines" for use by federal regulatory agencies in the risk assessment process. Called "The Red Book," because of the color of the cover, an emphasis was placed on the need to separate the scientific and the policy aspects of risk assessment. Risk assessment was defined as: "the use of the factual data base to define the health effects of exposures of individuals or populations to hazardous materials or situations." It organized the risk assessment processes into four areas. *Hazard identification* is the determination of the effects of the chemical in

exposed animals or people. *Dose-response assessment* evaluates the exposure conditions under which these effects are observed. *Exposure assessment* estimates the amounts of the chemical present in the workplace, home, and general environment. The fourth component, *risk characterization* combines information on the exposure and dose-response assessment to estimate the risk level for specific individuals, groups, or populations. Various default methods are used in risk and safety assessments. These defaults are used to circumvent lack of detailed knowledge of the shape of the dose-response curve. In general, these policy-driven defaults are designed to provide a conservative basis for estimating likely risks in exposed humans. This conservatism reflects an attempt to ensure adequate protection of members of the population in the absence of knowledge.

The reliance on these defaults could be reduced by improved understanding of the shape of the dose-response curve in regions of low incidence. The major role of quantitative systems modeling in risk assessment is for enhancing dose-response assessment and in assisting in the various extrapolations, especially in understanding the biological factors that determine the shape of dose-response curves for adverse responses at low levels of incidence, that is, in regions of the dose-response curve where the probability of response in a population is small, and between animal species. The quantitative relationships among these biological factors are determinants of incidence-dose relationship for toxic responses. Mathematical models of various kinds can integrate this biological information to predict (calculate) incidence for various exposure situations. These models need to be developed in such a fashion to be concordant with biology and the chemistry of the compound in the biological system.

1.1.4 Quantitative Models in Risk Assessment

Both qualitative and quantitative inputs are required in the process of conducting safety/risk assessments. The qualitative studies are important for cataloging information about: (i) hazard, that is, the possible effects of a compound irrespective of exposure considerations; (ii) mode-of-action; (iii) progression, that is, the steps connecting initial interactions on to impaired function; and, (iv) susceptibility, that is, the inter-individual differences that may make any one person more affected by exposure than another (e.g., gender, age, genetics, pre-existing disease).

Dose-response models were developed in the 1930s to assess responses in a population of animals treated with chemical, such as efforts to estimate the lethal dose in 50% of an exposed population (the LD50) or the effective dose for some response in 50% of the population, the ED50. Here a tolerance distribution depicted a quantal "either–or" response of individuals to chemical treatments. Incidence (number responding divided by number treated), either as a frequency distribution or as a cumulative response, was plotted against dose. In incidence-dose (ID) models, each member of the population has some dose sensitivity at which they respond to the test compound, and the "sensitivity" of individuals is considered to be distributed normally or log normally with tissue dose (Figure 1.1). In these analyses, the sensitivity of individuals and the variability in the population are estimated from the ID relationship, not from the underlying chemistry that describes delivery of chemical to target tissues or from the biology of the interactions of the chemical with specific cellular targets. This level of detail was unattainable in the 1930s; it is attainable in the twenty-first century.

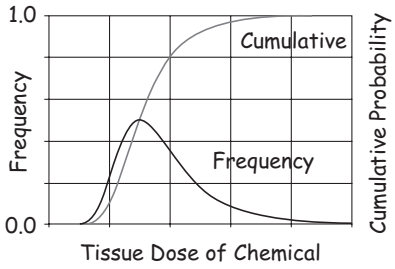

Figure 1.1 *Biological responses were initially described by tolerance distributions within a population. Incidence-dose curves were generated by assuming that each individual in the population had some sensitivity or tolerance for expressing the biological responses. The frequency distribution for the response was the proportion of the population responding to a given tissue dose; the cumulative curve integrated the response from zero (no individual in the population had responded) to 1.0 (all members of the population had responded). In this figure, the distribution is represented as a log normal distribution, with skewing of the distribution towards high doses when plotted on a linear dose scale, as shown here. Andersen* et al. *(2005a), reprinted with permission from Elsevier. Copyright 2005.*

Quantitative models have been developed to predict the relation between exposure and tissue dose (i.e., to describe the delivery of test molecule/metabolites to target tissues) and between tissue dose and tissue response (i.e., to describe the manner in which the molecular and cellular interactions of toxic compounds cause perturbations that are sufficiently large and sufficiently prolonged to lead to an adverse response). The broad categorization of these models breaks down into pharmacokinetic (PK) models for dosimetry and pharmacodynamic (PD) models for response. Pharmacokinetics has broadly been defined as what the body does to the compound; pharmacodynamics is what the compound does to the body. In order to be confident in the predictions/calculation from any model structure, these models themselves need to be as biologically realistic as possible without adding extraneous detail. Physiologically based (PB) models, such as physiologically based pharmacokinetic (PBPK) and physiologically based pharmacodynamic (PBPD) models, tend to be more firmly grounded in principles of biology and biochemistry (Reddy *et al.*, 2005) than are conventional, compartmental models. Biologically based dose-response (BBDR) models predict expected incidence of adverse responses for varied exposure situations and, by their nature, combine PBPK and PBPD approaches. These model structures can provide important tools for improving risk and safety assessments.

Starting in the 1980s there was considerable activity to create BBDR models for organism responses, including cancer and reproductive toxicity. These approaches had structures where dose led to alterations in cell growth, cell death, and mutation. Less detail was provided about the manner in which test chemicals altered cellular responses leading to the macroscopic changes in cell growth, differentiation, and mutation. Over the past decades with the growth of tools in cell and molecular biology, we now have the ability to query components of cellular, organ, and organism-level processes with exquisite detail, taking advantage of various new technologies, broadly referred to as "omics," including components of genes, gene products, proteins, and various small molecules. This diverse

array of data, however, needs to be organized quantitatively to create information, that is, to discover how the parts of the system are organized and controlled both in time and in space to provide biological functions, and how chemical exposures may perturb these functions. In this regard, "systems approaches" represent the attempt to quantitatively organize information across multiple levels of biological organization to unravel the manner in which biological functions are produced from simpler molecular, cellular, and organ interactions.

1.1.5 Systems Approaches in Pharmacokinetics

Compartments in PBPK models correspond to discrete tissues or to groupings of tissues with appropriate volumes, blood flows, and pathways for metabolism of test chemicals (Bischoff and Brown, 1966; Leung, 1991). Pertinent biochemical and physicochemical constants for metabolism and solubility are included in each compartment with routes of dosing described maintaining the proper relationship between the dosing site and the overall physiology of the portal of entry. The time-course behaviors of chemical throughout the body are then accounted for by equations that form the basis of the PBPK model and permit introduction of multiple routes, if necessary, for specific exposure situations. PBPK models have developed for a wide variety of compounds, associated with diverse toxicological outcomes (Reddy *et al.*, 2005). PBPK modeling is an example of a systems approach applied at the level of cells, organs, and organisms to integrate the mechanisms of distribution and interactions of environmental chemicals and drugs in the body. Two chemical engineers, Drs Kenneth Bischoff and Robert Dedrick, who first incorporated engineering principles, physiology, chemistry, and biochemistry into a computer modeling platform to predict kinetics (Bischoff *et al.*, 1971; Dedrick, 1973) are generally credited with being the pioneers in use of contemporary PBPK modeling.

These PBPK models do integrate information across multiple levels of organization, especially when describing the interactions of compounds with molecular targets, processes that include reversible binding of ligands to specific receptors, for example, methotrexate (Bischoff *et al.*, 1971) or dioxin (Leung et al., 1990) and the adduction of proteins or DNA by reactive parent chemicals or their metabolites in various tissues, for example, ethylene oxide (Krishnan *et al.*, 1992) or acrylonitrile (Gargas *et al.*, 1995). The goal in PBPK modeling is to integrate molecular, cellular, organ level, and organism-level processes to account for the time-courses of chemicals, metabolites, and bound complexes within multiple organs in the body. To a large extent, the main emphasis with these PBPK models is to account for the major determinants of the distribution and elimination of compounds without describing every physical chemical process involved in transport and storage of chemical in every tissue. Following the law of parsimony, making the model only as complex as possible for its intended use requires purposeful simplification in model construction. Increasing levels of detail in specific tissues can always be included in these models as more information becomes available on chemical disposition from specific experiments.

In the early application of PBPK modeling with environmental chemicals, for instance, many examples were quickly discovered where the kinetics were of necessity linked to dynamics. With methylene chloride, a metabolite, carbon monoxide, binds heme proteins and this interaction had to be taken into consideration (Andersen *et al.*, 1991). Other examples included dioxin inducing a dioxin-binding protein in liver (Leung *et al.*, 1990; Kohn *et al.*,

1993), ethylene dichloride depleting glutathione, thereby altering conjugation rates with the parent compound (D'Souza, Francis, and Andersen, 1988), and *trans*-dichloroethylene acting as a suicide inhibitor to reduce rates of oxidative metabolism (Lilly *et al.*, 1998). While adding a degree of complication to the PBPK description for these individual materials, the PBPK modeling approach was sufficiently versatile to accommodate these biological interactions and provide good descriptions of dose to target tissues for parent compounds and key metabolites.

These models support calculation of measures of the concentrations of test chemicals and metabolites reaching target tissues in the body during various exposures (Gentry *et al.*, 2002). They have been applied for both chemicals not normally found in the body (xenobiotics) and for chemicals found in the body that are toxic in conditions of excess or deficiency, such as the essential element, manganese (Nong *et al.*, 2009). Risk assessment application has also required development of tools for variability, uncertainty, and sensitivity analysis (Allen, Covington, and Clewell, 1996; Clewell and Andersen, 1996; Clewell, 1995), and new technologies associated with Bayesian methods and Markov Chain Monte Carlo tools have appeared to assist in estimating parameters in these PBPK models. In addition, dose metrics derived from PBPK modeling are also commonly used in deriving benchmark values (Barton *et al.*, 2000). These methods involve fitting a variety of empirical mathematical models to dose-response data to estimate a dose, with attendant confidence intervals, that is associated with predetermined benchmark response (BMR), for example, a 10% alteration in the target response (Allen, Covington, and Clewell, 1996).

1.2 Linking Doses and Response

To link exposure and outcome for toxic compounds, toxicology and risk assessment have focused on the exposure-dose-response relationship for the past 25 years (Figure 1.2). PBPK models provide greater detail on the steps up to tissue interactions, including binding of reactive molecules with cellular macromolecules or recognition of chemical structures by reversible binding of the xenobiotic to cellular receptors that regulate key cell signaling pathways. The measures of tissue dose that are more closely aligned with tissue responses are called dose metrics (Andersen, 1987). These measures are preferred as the basis for the dose-response portion of chemical risk assessment. The steps linking these dose metrics to

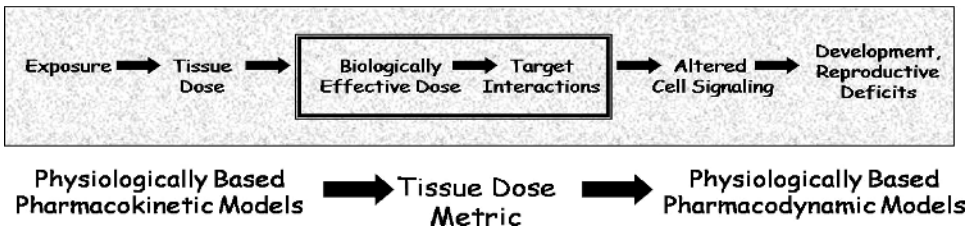

Figure 1.2 *The Linear Exposure-Dose-Response Paradigm for Organizing Toxicology Research and Testing for Risk Assessment Applications as defined in the 1980s. Andersen* et al. *(2005b), reprinted with permission from Elsevier. Copyright 2005.*

response are part of the pharmacodynamics (PD) of the response to chemical exposures. In this manner, dose metrics are the equivalent of a "biologically equivalent dose" (BED) and link active forms of the chemical at target tissues to the response of concern via the mode of action. Through the development of the PBPK modeling, tissue dose metrics have been linked with integrated cellular level responses, for example, cancer, cytotoxicity, and so on, to assist in risk assessments and guide various extrapolations (Clewell and Andersen, 1985). However, PD models have been more empirical, making use of simple effect compartments with responses correlated with blood or tissue concentrations of active chemical. Other PD approaches, called biologically based dose-response (BBDR) modeling, include two-stage clonal growth models for carcinogenesis and cell growth based models for developmental toxicology. These BBDR models were developed to assist with risk assessment (Moolgavkar and Luebeck, 1990; Leroux *et al.*, 1996; Whitaker, Tran, and Portier, 2003). In these descriptions, adverse endpoints are a function of compound-related alterations in cell replication, apoptosis, and mutation rates. In general, the parameters in the BBDR models have not yet been described with respect to the effects of chemicals on specific cellular signaling pathways or the interactions among signaling pathways. 5-Fluorouracil (5-FU) is arguably the best example of a BBDR model for developmental toxicity where tissue concentrations are linked to enzyme inhibition, impaired nucleotide synthesis, altered DNA synthesis, and, finally, developmental anomalies (Lau *et al.*, 2001; Setzer *et al.*, 2001). As in all response models, a challenge with 5-FU is the difficulty in first providing an adequate description of the underlying biology that is being affected by the compound. The problem in describing biology is a particular issue for development where processes and structures are changing rapidly and consecutive developmental landmarks are critically dependent on completion of earlier steps.

1.2.1 Systems Biology and Dose-Response Assessment

As noted earlier, PBPK models represent a "systems approach" to the physiological and biochemical levels. The ability to apply more integrated systems approaches to tissue response modeling has been seriously impeded by limited knowledge before the expansion of methods in cell molecular biology over the past two decades. These systems biology approaches integrate diverse data across various "omic" technologies and biological organization to understand how these various components lead to specific biological functions. Today, these high throughput, broad coverage technologies – such as, genomics, transcriptomics, proteomics, and metabonomics – are generating molecular "parts lists" for the components of cells, tissues, organs, and all the way to the organism level. In a perspective in *Science* at the turn of the millennium, Lander and Weinberg (2000) discussed the implications of the information generated by "genomics" technologies in providing a more complete understanding of biology:

> *The long-term goal is to use this information to reconstruct the complex molecular circuitry that operates within the cell – to map out the network of interacting proteins that determines the underlying logic of various cellular biological functions including cell proliferation, responses to physiologic stresses, and acquisition and maintenance of tissue-specific differentiation functions. A longer term goal, whose feasibility remains unclear, is to create mathematical models of these biological circuits and thereby predict these various types of cell biological behavior.*

Current initiatives in computational systems toxicology emphasize an iterative process, with recurrent steps through laboratory experiments and computer modeling, to create an understanding of the manner in which the components of biological systems are organized in order to produce these circuit elements that regulate biological function. Perturbations of these biological processes by environmental stressors, including chemicals and drugs, can lead to adverse responses (toxicity), restoration of normal function to a compromised tissue (drug efficacy), or control of biological processes, such as occurs with use of hormonal therapies in post-menopausal women. Computational systems biology is now an intense area of activity providing key information for understanding cell behavior. Some of these approaches were highlighted in the focused series of papers in *Nature* in 2002 (Kitano, 2002; Surridge, 2002). More recent contributions outline contributions of computational systems biology to understanding the circuitry controlling biological responses and stress pathways (Alon, 2006, 2007).

Toxicology and pharmacology are disciplines at the interface of chemistry/pharmacokinetics (primarily embedded in the vertical component from exposure to perturbation) and biology/pharmacodynamics (primarily captured by the horizontal chain from inputs to normal biological function – Figure 1.3). With the rapid advances in quantitative understanding of many biological processes and responses to perturbations, toxicology modeling can focus more directly on the underlying biology rather than simply the perturbations

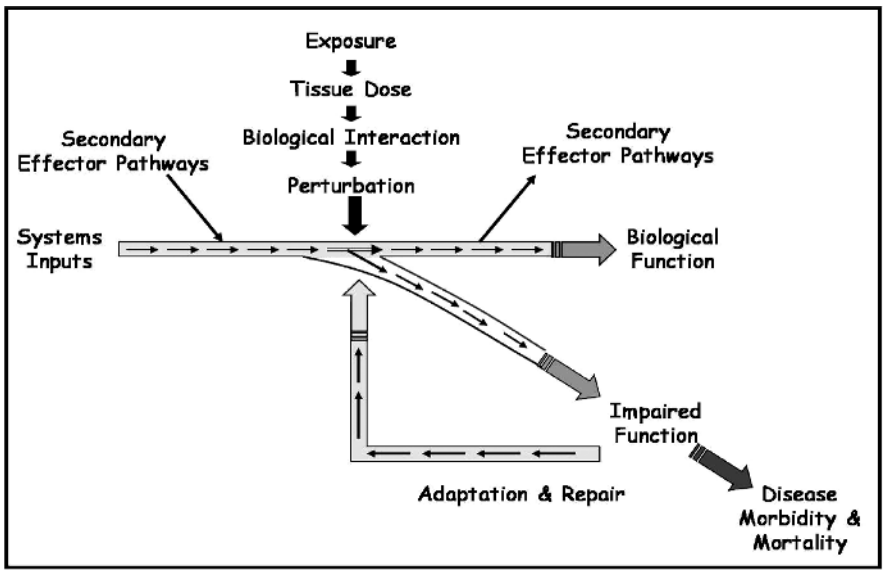

Figure 1.3 *The Maturing Exposure-Dose-Response Paradigm for Toxic Responses is related to Perturbations of the Normal Control Processes in the Cell by Toxicant Exposures. Low doses are largely without functional consequences; intermediate doses activate adaptive stress responses with attendant homeostatic controls; and, high enough doses lead to overt toxicity. Increasing dose leads to progress through several dose-dependent transitions. Andersen* et al. *(2005b), reprinted with permission from Elsevier. Copyright 2005.*

following chemical exposure. When the exposure-dose-response is recast with intersection of chemical perturbation with normal biology, toxicity and efficacy become situated at the intersection of chemical action with the underlying biology. We are now moving to a second-generation of systems approaches in PK and PD modeling. This new initiative will include simulation models with increasingly detailed descriptions of biology derived from new technologies coupled with the expansion of current modeling tools that focus on signaling pathways affected by chemical exposures and drug treatments.

1.2.2 Circuits, Networks and Signaling Motifs and Toxicity

Cells consist of multiple interacting modules, with various feedback controllers. These control modules have been primarily examined through detailed experimental work prokaryote cell systems. These simplified signaling circuits have been described by mathematical models to capture the behavior of the control networks. Using the variety of tools from molecular biology, simple prokaryotic cells have in some cases been engineered with specific circuit elements, including, biological oscillators, switches, amplifiers, and so on. These artificial constructs then can be queried by laboratory experiments and the results used to construct detailed models for the underlying circuitry controlling these behaviors (Hasty, McMillen, and Collins, 2001, 2002; Guet *et al.*, 2002; McMillen *et al.*, 2002; Tyson *et al.*, 2003).

Computational models have evaluated the protein networks within cells, the control of these networks by the component circuitry, and uncovered the logic of cellular responses affected by these networks (Davidson *et al.*, 2002; Ferrell, 2002; Alm and Arkin, 2003). Among these control processes are activation of latent networks, silencing of active networks, and modulation of network function. The circuitry is regulated by various input signals to the cells. Input signals, including endogenous compounds, such as hormones and various exogenous compounds, are capable of activating cell signaling networks to modulate both control circuitry and affect downstream genetic networks. Cell cycle control has been extensively modeled in eukaryotic organisms and represents a generic process that is common across biological organisms (Tyson, 1999; Tyson *et al.*, 2003). Progress in understanding similar common signaling themes (Ray, Adler, and Gough, 2004; Schlessinger, 2004) has increased the appreciation of the manner in which modular components are used to achieve and control a host of cellular outputs/functions.

Xenobiotics have effects on tissues in biological systems through two general processes – receptor-mediated recognition of the three-dimensional structure of the compound – or stress pathways, where the cells respond to chemical reactivity and stress through negative feedback control processes to maintain homeostasis. These interactions perturb cell circuits and modulate cellular functions. This classification of response pathways is especially relevant for so-called receptor-mediated toxicants that interact in the body with specific endogenous receptors. Receptor-mediated toxicants have the potential to cause toxicity by mimicking endogenous signaling molecules, leading to over-stimulation of natural circuits. Alternatively, compounds of this class may cause toxicity because they are competitive inhibitors of natural receptor-mediated functions. Competitive inhibitors can bind with endogenous receptors, but cause failure of activation of important circuits in the presence of normal signals. Inhibitory compounds with a receptor-mediated mode of action can lead to diseases associated with deficiencies of signaling molecules and circuit activation.

The report from the National Academy of Sciences, Scientific Frontiers of Developmental Toxicity and Risk Assessment (NRC, 2000), outlined the suite of known intercellular signaling motifs and their roles that these pathways play in development. This suite of signaling pathways represents potential targets that could lead to toxic responses if they are sufficiently perturbed by chemical exposures, that is, adverse responses could ensue if these pathways were inhibited or over-stimulated at times during development.

1.2.3 Modeling Cellular Response Pathways

Cell signaling pathway model code is similar to the sets of ordinary differential equations used in most PBPK models. There are some new challenges because the low copy number of genes, transcripts, and proteins may require stochastic simulation. Despite these difficulties, several mammalian signaling pathways have been modeled – including platelet derived growth factor (PDGF) pathway (Bhalla, Ram, and Iyengar, 2002) in mouse 3T3 cells and tumor necrosis factor-alpha (TNF-α) signaling through the nuclear factor kappa B (NF-κB) pathway in mouse fibroblasts (Hoffmann *et al.*, 2002; Cho *et al.*, 2003). The equations in these models represent groups of reactions within a cell and require estimation of multiple parameters in the biochemical steps embedded within the signaling networks. Models based on series of differential equations have also been augmented by Boolean approaches that use on-off logic to increase the model coverage on intracellular reactions, without necessarily increasing the numbers of parameters that have to be estimated for all the protein–protein interactions involved in the signaling networks (Bolouri and Davidson, 2002).

The huge diversity of cellular responses and interactions can be broken down and categorized in a much smaller set of functional circuits – positive and negative feedback, feed-forward loops, and so on. While there are many discrete signaling modules within any cell, the process of careful modeling of prototype circuits that allows dissection and modeling of individual signaling modules should eventually encourage a modular approach to understanding more integrated biological pathways (Hartwell *et al.*, 1999). Progress in applications of these modeling strategies in toxicology and risk assessment is likely to come from model development for a series of prototype compounds. These compounds would be selected based on knowledge of the key signaling pathways with which they interact. The computational systems biology models of the response pathway could then focus on the circuits giving rise to pathway function, allowing improved mechanistic understanding of the dose-response behavior of the circuit serving as a primary focus of model development.

The increasing focus on cell response pathways for toxicity testing (NRC, 2007) is likely to spur greater interest in computational systems biology for evaluating the dose response for perturbations and for activation of adaptive responses. Along with a fairly large group of modern computational biologists, Alon (2006) has emphasized the pattern of recurring motifs and commonality of motif functions that are present in larger scale networks. Nonlinear processes associated with positive feedback, double negative feedback, negative feedback homeostasis, and coherent and incoherent feed-forward loops are increasingly being analyzed using engineering principles to understand cell function and how the intrinsic cell circuitry is designed to achieve specific functions (Alon, 2007). A course on computational systems biology and dose-response modeling at The Hamner Institutes for Health Sciences has outlined the key ideas and focused on both numerical simulation and on understanding the nonlinear dynamics that are the basis of complex

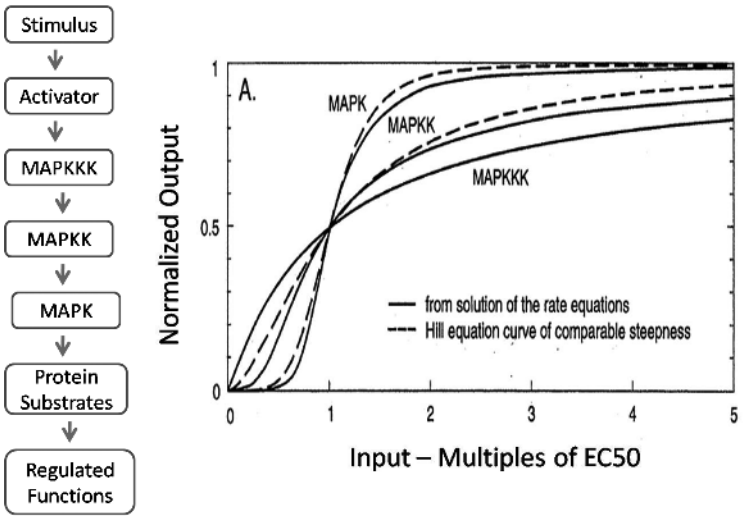

Figure 1.4 *The mitogen-activated protein kinase (MAPK)cascade generates nonlinear dose relationships for cellular responses. This signaling motif has three sequential kinase partners – MAP kinase kinase kinase (MAPKKK), MAP-kinase kinase (MAPKK), and MAP kinase (MAPK). The steepness of dose response increases through each component of the cascade. Reprinted with permission from Huang* et al. *Copyright 1996, National Academy of Sciences, USA.*

cellular responses to chemical and environmental stressors (http://www.thehamner.org/education-and-training/current-course-offerings.html).

Cell response modeling, more than PBPK or PBPD simulation models, has created increasing attention on the characteristics of nonlinear dynamics rather than on numerical solution of series of differential equations (Strognatz, 1994). Key topics for cell signaling include ultrasensitivity (Ferrell and Machleder, 1998; Ferrell, 2002), bistability (Xiong and Ferrell, 2003), and the use stochastic differential equation solvers that account for noise and randomness in cellular reactions that involve small numbers of participating molecules (Kaern *et al.*, 2005; Gillespie, 1976). One ubiquitous ultrasensitive motif in eukaryotes is the mitogen-activated protein kinase (MAPK) cascade (Johnson and Lapadat, 2002) that participates in a variety of cell response pathways (Figure 1.4). Through activation of the sequential kinase components, these motifs generate ultrasensitive behaviors, with equivalent for steepness for Hill-curves with n-values of greater than 5.0 for the MAPK itself (Huang and Ferrell, 1996). These ultrasensitive motifs coupled through positive feedback produce bistability, that is, true switches, in cellular response behaviors (Xiong and Ferrell, 2003).

A common discussion among the community working on quantitative modeling of complex responses is whether the models should be built bottom-up (focusing on the initial interactions and going on to adverse responses) or top-down from the adverse consequences at the organism level and piecing the path back to uncover the key portions of the toxic response. Increasingly, it appears that real progress in quantitative modeling of toxic responses is likely to arise from a middle-out approach, where the primary responder is the cell, with model structures focusing on how cells respond to perturbations and how excessive perturbations cause cascades of cellular pathway activation, for example,

anti-oxidant stress, up-regulation of anti-oxidant response genes, inflammation, and, finally, apoptosis (Nel *et al.*, 2006). The field of quantitative systems modeling for toxicology and risk assessment is emerging as a critical area for informing risk and safety assessment with environmental chemicals and drugs. Zhang *et al.* (2009), for instance, have examined dose-response relationships for reactive intermediates where liver concentration of reactive metabolites from Phase I enzymes is controlled by feed-forward activation of phase II and phase III enzymes. Feed-forward homeostatic control can produce complex, and in some cases, hormetic dose response. Integration of biomedical engineering concepts with the biology of cell signaling should provide theoretical justification for dose-response curves with a wide variety of shapes and allow consideration of dose-dependent transitions as key components of dose-response assessment strategies (Slikker *et al.*, 2004).

1.3 Summary

Quantitative modeling in toxicology dates back to the development of dose-response methods for estimating effective doses using probit analyses for quantal responses. Major advances increased our ability to understand dose to tissues through pharmacokinetic (PK), and, especially, physiologically based pharmacokinetic (PBPK) modeling. PBPK models have spurred growth of sensitivity, variability, and uncertainty analysis; they forced development of statistical tools for assessing goodness-of-fit of models to diverse data sets; and they instigated strategies for using measures of internal dose in risk assessment through linkage with benchmark dose (BMD) models. Response modeling matured more slowly due to lack of detailed understanding of the biological processes leading to organism level responses. Response models for cytotoxicity, cancer, and developmental toxicity have focused on cell level responses and relied on admixtures of mechanistic and empirical structures. Models for precursor responses related to chemical adduction of protein and DNA and enzyme inhibition retained more fidelity with the chemistry of test compounds and the biology of the precursor interactions. Looking forward, rapid development are expected in continued expansion of PBPK models at the organism, organ, and cell levels, and in computational systems biology modeling of intra- and intercellular signaling, leading to more biologically relevant dose-response models for cell and tissue level responses. Other advances in bioinformatics and high data content methodologies will drive new dose-response modeling approaches that organize genomic results on the basis of the classification of gene families affected by chemical treatment (see for example, Thomas *et al.*, 2007; Andersen *et al.*, 2008) that are more difficult to predict. It is hoped that the chapters and examples in this volume will spur further development of quantitative modeling in toxicology and continue the improvement of chemical risk assessment by encouraging quantitative, biological modeling approaches for toxicology and for risk assessment decision making.

1.4 Acknowledgment

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