Quantitative Modeling in Toxicology

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Preface

The goal of most toxicology studies is to help reach some conclusion about likely risks posed to humans or to other species from chemical exposures. Test results, both from in vivo and in vitro studies, require various forms of extrapolation to make risk predictions for specific exposures and in specific populations. Over the past 50 to 60 years, a variety of modeling tools have emerged that help in describing toxicological processes quantitatively and in making these extrapolations. These quantitative models have substantially improved our understanding of human exposure, pharmacokinetics, mode of action and toxic responses associated with chemicals. In recent years, toxicology, as true for other biological disciplines, has also been enriched by the new tools from genomic biology and by the increasing emphasis on computational systems biology for describing cell and tissue function.

The opportunity now exists for toxicology to transition from a qualitative science cataloging responses in various animal species to a discipline capable of quantitatively describing key mechanistic processes that determine dose-response behaviors for animal and human responses. Quantitative modeling in toxicology includes approaches that simulate (i) exposure and disposition of chemicals in the body, (ii) biochemical interaction between toxic moiety and target tissues, (iii) molecular and cellular alterations emanating from the initial interactions; and (iv) adverse responses at the organ or organism level. Properly developed, these quantitative mechanistic models can provide unambiguous, testable statements of working hypotheses regarding chemical uptake, biochemical interactions, and the initiation and progression of toxicity in the exposed organism.

Integration of quantitative tools within experimental design, data collection and analysis as well as risk assessment applications is more important than ever. Specifically, these tools are important (i) for conducting scientifically sound extrapolations of dosimetry and responses for risk assessment purposes, (ii) for refining/reducing animal use in toxicology studies by facilitating the development of new, novel and efficient experiments, and (iii) for creating a framework with which to integrate the various observations (exposure, dose, mechanism, response) at a quantitative level. Despite the growth in interest in these quantitative models in toxicology, there are few resources to serve as a guide in learning more about these tools. The two of us, along with other colleagues, have taught modeling courses at our respective institutions through lectures and computer demonstrations. In these courses, we have also felt the need for a concise overview of quantitative modeling in toxicology and began discussions leading to this book.

Quantitative Modeling in Toxicology now brings together contributions from key scientists on the modeling of exposure, tissue dose, tissue interaction and toxicological responses. Chapters 2-5 describe the quantitative models of pharmacokinetics of individual chemicals
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and mixtures. Chapters 6 through 11 describe models for toxicant-target tissue interaction. Chapters 12 through 15 describe models for cellular, organ, and organism responses. The simulation models of toxic effects based on the toxicant-target interaction models and mode of action information are highlighted with specific examples. Finally, Chapters 16 through 21 present the approaches, tools and challenges regarding the application and evaluation of quantitative models for exposure and risk assessments. Based on the breadth of the material in these chapters, this book should serve as an initial reference for toxicologists and risk assessors who are interested in developing quantitative models for a better understanding of dose-response relationships.

The process of simulation modeling requires writing computer code to represent the biological systems and the consequences of exposure. The models are written in a computer language and then solved by numerical integration. The examples throughout the book use a variety of commercial software, including ACSL®, BERKELEY MADONNA®, MATLAB®, EXCEL® and MEGen®. We do not endorse any particular software and did not require our contributors to use any particular software. In general, the source code developed in one language can be easily recoded into alternative language. Code for running various models in the book have been included in specific chapters and made available for download from the publisher. The text files for these models are intended to assist the interested reader in developing models for their own use or for use for instruction. We would appreciate comments from our readers about the value of these models for learning more about quantitative modeling in toxicology.

Needless to say, the completion of this work was in large part due to our group of talented authors. Thanks to all! We also thank Richard Davies of Wiley for his enthusiasm and cooperation throughout this project as well as Michelle Gagné and Mathieu Valcke of Université de Montréal for editorial assistance.

KK and MEA
About the Editors . . .

**Kannan Krishnan, PhD, DABT, FATS** is Professor of Occupational and Environmental Health at Université de Montreal and Director of the Inter-University Toxicology Research Center (CIRTOX), Montreal, Canada. An expert in the areas of PBPK modeling, chemical mixture toxicology and health risk assessment methods, Dr. Krishnan has held visiting scientist/faculty appointments at the Karolinska Institutet, Sweden (2004), Toxicology Excellence for Risk Assessment (TERA, Cincinnati, OH) (2007) and Environmental & Occupational Health Sciences Institute of UMDNJ-Rutgers University, NJ (2007). He received the *Veylian Henderson Award* of the Society of Toxicology of Canada (2000) and the SOT Board of Publications Award for the *best paper in Toxicological Sciences* (2003) for a land-mark publication on the PBPK modeling of metabolic interactions and health risk assessment of chemical mixtures. He was a significant contributor to the U.S. EPA report “Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment” (2006) and IPCS/WHO guidance document “Characterization and application of PBPK models in support of risk assessment” (2010).

**Melvin Ernest Andersen, PhD, DABT, CIH, FATS** is Director, Program in Chemical Safety Sciences at The Hamner Institutes for Health Research, Research Triangle Park, NC, U.S.A. He is reknown for his career contributions in developing quantitative models of the dosimetry and effects of drugs and toxic chemicals as well as applying these models in safety assessments and quantitative health risk assessments. Through short-courses in quantitative modeling in toxicology, Dr Andersen has trained several hundred toxicologists and risk assessors in quantitative modeling. An author or a co-author of 325 papers and 60 book chapters, he has received several awards for professional contributions; including the *Herbert Stokinger Award* (American Conference of Industrial Hygienists, 1988), the
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About the Book . . .

Quantitative modeling in toxicology is a must-read for those interested in computer simulation of the biological fate and effects of chemicals. It brings together a diverse group of experts in this area to provide the reader with the current state of knowledge regarding the modeling of dose, tissue interactions and tissue responses. Additionally, tools and approaches for model evaluation and application are described. Access to an electronic MODEL LIBRARY containing the source code for several dosimetry, toxicant interaction and toxicity models is included with the book in order to allow the interested reader to reconstruct the examples in the various chapters. This book will be of particular interest to graduate students, practicing toxicologists and risk assessors who are fascinated by the application of quantitative modeling approaches to simulate perturbations of biological systems upon exposure to xenobiotics.
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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 \textit{discrete} if the state variable does not depend upon the time variable (Bellomo and Preziosi, 1995); the \textit{continuous or dynamic} models describe the change in state variable over time. A model is \textit{deterministic} if its outcome is a direct consequence of...
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 ($C_0$) and elimination rate ($k$):

$$C_t = C_0 e^{-kt}$$

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.
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Cumulative Frequency

Tissue Dose of Chemical

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

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**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._
