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Improving Risk Assessment: Toxicological Research Needs[1]

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ABSTRACT

A workshop convened to define research needs in toxicology identified several deficiencies in data and methods currently applied in risk assessment. The workshop panel noted that improving the link between chemical exposure and toxicological response requires a better understanding of the biological basis for inter- and intra-human variability and susceptibility. This understanding will not be complete unless all life stages are taken into consideration. Because animal studies serve as a foundation for toxicological assessment, proper accounting for cross-species extrapolation is essential. To achieve this, adjustments for dose-rate effects must be

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improved, which will aid in extrapolating toxicological responses to low doses and from short-term exposures. Success depends on greater use of validated biologically based dose-response models that include pharmacokinetic and pharmacodynamic data. Research in these areas will help define uncertainty factors and reduce reliance on underlying default assumptions. Throughout the workshop the panel recognized that biomedical science and toxicology in particular is on the verge of a revolution because of advances in genomics and proteomics. Data from these high-output technologies are anticipated to greatly improve risk assessment by enabling scientists to better define and model the elements of the relationship between exposure to biological hazards and health risks in populations with differing susceptibilities.

Key Words: susceptibility, life stages, pharmacokinetics, dose response, exposure assessment, genomics.

INTRODUCTION

Considerable progress has been made over the last 20 years in defining discrete components that affect relationships between exposure circumstances and biological effects. However, toxicology must move toward better characterization and understanding of the key cellular and molecular alterations that are responsible for adverse effects observed in experimental animals and humans. To achieve this goal, research is needed to address several deficiencies in data and methods currently applied in risk assessment. To improve the link between chemical exposure and toxicological response, the following issues must be considered: (1) increased understanding of inter- and intra- individual variability in susceptibility with special attention to susceptibility during all life stages, (2) accounting for factors that affect cross species extrapolation, (3) adjusting for dose rate effects, (4) defining toxicological responses at low doses, (5) making use of continuous as well as quantal data from toxicological responses, (6) developing better response data from short-term exposures, (7) addressing exposures to chemical mixtures and by multiple exposure routes, and (8) refining uncertainty factors with reliable experimental data. Improvement in most of these areas requires identification and quantification of molecular and cellular biomarkers along the critical pathway between exposure to an agent and clinical or functional expression of toxicity. Improvement also depends on developing biologically based dose response (BBDR) models that can link exposure and biological response in a physiologically realistic framework. The framework must account for the time- and dose-dependent delivery of the toxic form of the agent to its biological target (kinetics) and the time- and dose-dependent changes in the biological system that lead to an adverse response (dynamics).

INTER- AND INTRA-INDIVIDUAL VARIABILITY IN SUSCEPTIBILITY

Traditionally, chemical risk assessors and their methods have focused on defining qualitative and increasingly quantitative relationships between exposure to toxicants and adverse effects. A risk assessor's ability to establish this connection has been hampered by inter-individual variability (Hattis 1996). Although much uncertainty in risk assessment is attributable to absent or inconsistent data, this source of

uncertainty theoretically can be addressed by filling identified data gaps. In contrast, inter-individual variability can only be addressed by understanding the underlying basis for the variability and then applying new methods that enable the incorporation of this information into risk assessments. A major source of uncertainty in risk assessment is how responses to chemicals and physical agents vary not only among individuals but within an individual under changing circumstances. Gender, race, ethnicity, lifestyle, genetic predisposition, and age (conception to senescence), are factors that must be considered in risk assessment, as they can contribute to variation among individuals in disease outcome resulting from environmental insult (Perera 2000). Age, life-style changes, reproductive status, drug use, and previous exposures, among other factors, can also contribute to response variation within an individual over time.

While experimental toxicologists generally design studies to control for inter-individual variability, epidemiologists routinely include corrections for confounders or effect modifiers such as smoking, alcohol use, diet, gender, race, and age. When appropriate, this information is included in risk assessments with the ultimate goal of reducing uncertainty. Although gender, age, and diet have been addressed in experimental animal studies, race, ethnicity, and lifestyle factors are not easily addressed. As a consequence, risk assessments must rely solely on human data to assess the contribution of these factors.

Increasingly, epidemiological studies have attempted to identify genetic polymorphisms that may explain in part inter-individual variations. Genetic polymorphisms that appear to predispose individuals to cancer have received the most attention. The most extensively studied susceptibility factors are the genetic variations in Phase I and Phase II xenobiotic-biotransformtion enzymes (Perera 2000). Investigation of genetic variants in cytochrome P450 (Ishibe *et al.* 1997; Mollerup *et al.* 1999), glutathione *S*-transferase, and *N*-acetyltransferase (Trizna *et al.* 1998) have contributed greatly to an understanding of the source of variation among individuals in terms of their response to chemical carcinogens. Altered expression of these enzymes because of inherited polymorphisms or differences in the levels of enzyme induction can lead to different abilities to activate or detoxify xenobiotics and thereby alter a person's risk to disease.

Biotransformation and metabolism are not the only concerns. Also important are inherited variations that predispose a person to cancer. Receiving considerable attention in the lay press are the breast and ovarian cancer susceptibility genes BRCA1 and BRCA2 (Brody and Biesecker 1998). These genes are believed to be important for tumor suppression. Furthermore, BRCA1 has been linked to a DNA repair protein (Gowen *et al.* 1998). Variation in DNA repair can affect a person's risk from agents that directly or indirectly damage DNA. Increased understanding of these sources of variation is needed as they have the potential to identify persons at increased risk from exposure to a toxicant.

Completion of the sequencing of the human genome and new technologies to evaluate gene expression, have given rise to greater opportunity to use this molecular information in risk assessment. However at present, the understanding is inadequate to determine when and how incorporation of data on genetic polymorphisms may affect a risk assessment and by implication, influence risk-based policy. To determine this, it will be necessary to investigate the nature and magnitude of

the impact of incorporating this information into specific risk assessments. Specifically, research must investigate the extent to which a single or combination of genetic polymorphisms affects the toxicity of environmental and occupational exposures. Physiological and molecular methods can be used to establish the phenotypic significance of genetic polymorphisms in order to estimate the impact of genotype on response to toxicant exposure. This may provide a means of calculating the significance, for risk assessment, of genetic variances that encode measurable phenotypic differences.

SUSCEPTIBILITY DURING LIFE STAGES

Sufficient, valid scientific data now exist to assert that both pre- and postnatal exposures to a variety of toxic substances can deleteriously affect the health and development of neonates and young children. Indeed, there is basis for concern that such prenatal exposures can have life-lasting effects and can manifest impacts on later life stage function and behaviors. Such toxic substances include lead, methylmercury, PCBs, ethanol, and carbon monoxide, among others. Depending on the dose received by a fetus and the specific toxicant, health consequences can range from subtle toxicological changes in animal models, including neurobehavioral effects, to death following high exposures. One area of concern regarding adverse effects of exposure on reproductive health is occupational exposures to solvents (Taskinen *et al.* 1999; Plenge-Bonig and Karmus 1999). Several recent studies have reported on relationships between parental occupational exposures and risks of childhood cancer (Colt and Blair 1998).Critical for an evaluation of potential toxicological impacts throughout development is both knowledge of key developmental pathways and their potential susceptibilities to toxicants. This necessitates the availability of test methods to evaluate such potential impacts across development as well as evaluations with a sufficiently large database of test chemicals to validate such systems.

Testing approaches for early (prenatal and early postnatal) developmental toxicity have been available for some time, although gaps still exist in evaluation of certain endpoints after early exposures, *e.g.*, immunotoxicity, respiratory, cardiovascular, renal, and liver function, and cancer. Recent efforts to expand the exposure period for prenatal assessments reflect the knowledge that organ systems continue to develop beyond organogenesis. Further testing at later stages in development arises from concern that effects on development may be manifested much later in adulthood (Selevan *et al.* 2000). Effects of exposures in the periadolescent period have not been studied sufficiently. Yet many teenagers are in the work force and may be exposed to toxic chemicals, particularly in agricultural settings (Golub 2000).

At the other end of the spectrum, exposures in older age groups are not well-evaluated in current toxicity testing approaches. Only the 2-year chronic/carcinogenicity testing protocol includes exposures into later ages, and the effects of agent exposures may be masked or exacerbated by *ad libitum* diet, resulting in obesity, and its consequences. Diet restriction in rodents has clearly been shown to increase life span and reduce disease. Thus, testing of rodents at later ages in diet-restricted and unrestricted situations is needed to identify factors in the aging population that are important in the toxicity of various exposures. With the current trend toward an

aging workforce, this information may be useful in setting more appropriate exposure limits to protect the health of this segment of the population.

Recent studies have shown that isoenzymes of xenobiotic metabolizing systems are not expressed uniformly in developing humans (de Wildt *et al.* 1999). For example, human CYP3A7 is found prominently in human fetal liver but not in adult liver (Katida *et al.* 1985; Wrighton *et al.* 1988) whereas CYPs 1A2, 2B6, and 2C8 are expressed highly in children over 1 year old (Tateishi *et al.* 1997). Similar observations of variable expression of glutathione *S*-transferase have been reported (Tee *et al.* 1992). These results suggest that activation and detoxification capacity could be age- dependent. Thus, susceptibility of neonates to chemical exposure is likely to depend on the prevalence of activation/detoxification enzymes at the time of exposure.

Due to a lack of data on the exposure effects of human pregnant mothers to environmental or occupational agents and even less on their developing children, better use must be made of available animal data. Current regulatory study designs for examining potential effects on the developing fetus or neonate induced by environmental or occupational agents do not require any information on internal dose to the mother or fetal/neonatal dosimetry. Dosimetry information in the exposed mother, fetus, and neonate would certainly improve dose-response analysis. Most of the common laboratory species on which studies of developmental toxicity are conducted during these critical windows have a markedly different rate of development and timing of developmental stages compared with humans. Essentially, most rodents are born "premature" and many critical periods of development (e.g., brain and sexual differentiation) take place postnatally in rodents that occur *in utero* in humans. Thus, the effects of lactation on *in utero* exposure can be extremely different between humans and the animal species used for testing. Animal studies must be designed to capture the comparable critical windows of human development. To meet the need in risk assessment for linking temporal exposure information with temporally sensitive developmental processes, united pharmacokinetic and dynamic models must be developed. Such models are needed to provide more accurate evaluation of dose-response relationships for the dynamic processes occurring during critical periods in development (Faustman *et al.* 2000).

IMPACT OF LIFESTYLE FACTORS ON SUSCEPTIBILITY

The toxicity of many chemicals is regulated in part by enzymatic metabolism, whose activity is determined by a variety of intrinsic and extrinsic factors. The risk of toxicity depends on changes in these enzyme activities over time which may be influenced by both genetic and lifestyle factors. Lifestyle factors include such things as pharmaceutical use, alcohol intake patterns, and health status. Several drugs are known to be enzyme inducers, dietary factors and alcohol alter the activity of an important oxidative enzyme, CYP 2E1, which is involved in the metabolism of many environmental and occupational chemicals (Chien *et al.* 1997). The complex, time-dependent interactions of multiple lifestyle factors as determinants of chemical toxicity are often overlooked in normal standard toxicity testing venues. The pattern of chemical interaction is further complicated because many of the inducing compounds may also serve as inhibitors of degradative metabolism of workplace chemi-

cals. Therefore, research is needed to determine the conditions under which these interactions are likely to enhance toxicity, which members of the population are most at risk for these interactions, and which activities and lifestyle factors lead to higher risks from chemical exposures.

Research is needed that combines and integrates experimental studies in animals and studies with human tissues and/or human volunteers to create mechanistic models of the influence of lifestyle factors, enzyme induction, and temporally disparate exposures on metabolism and expected toxicity of environmental and occupational compounds in diverse populations. It will be essential to convey the method by which qualitative and quantitative inferences drawn from mechanistic animal studies can be extended to human populations and the method by which ancillary data from human tissues and/or human volunteer studies would support inferences from animal research. It will also be necessary to demonstrate the method by which these integrated studies provide improved quantitative characterization of the variability expected in a diverse human population in response to chemicals and to alterations in enzyme activities by lifestyle factors.

PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING

PBPK modeling is a powerful tool for extrapolating dosimetry across species, from high doses to low doses, and across various exposure durations. Recently, PBPK models have been developed that simulate the induction of various proteins over time (Santostefano *et al.* 1998; Andersen *et al.* 1993). Extending this technology to the development of the xenobiotic metabolizing system in the neonatal liver seems reasonable. PBPK models of lactational transfer have also recently been developed for several volatile organic chemicals, and these models have been used to predict exposure of infants as a result of occupational exposure of mothers to toxic chemicals (Fisher *et al.* 1997; Byczkowski *et al.* 1994). Data must be developed to model fetal/neonatal exposure to chemicals, through lactational transfer, where neonates may be more or less susceptible to chemicals that may or may not be bioactivated or detoxified by the maternal system. Experimental dosimetry data in adult and neonatal animals must be collected for use in the development of PBPK models to describe chemical kinetics in the adult and neonate for extrapolation of the results to the humans. Model systems should focus on key xenobiotic biotransformation enzymes that are expressed differently in neonates and adults.

BIOLOGICALLY BASED DOSE-RESPONSE (BBDR)MODELS

Biological models that address mechanistic steps linking exposure to adverse effects offer an objective, data-based approach to test biologically based hypotheses and to generate alternative hypotheses for laboratory testing (Leroux *et al.* 1996; Shuey *et al.* 1994). If mechanistic hypotheses are not adequately tested in an appropriate dose-response framework, then approaches to estimate occupational risks that rely on such hypotheses may simply be substituting one set of assumptions for another, and the latter set may not provide adequate health protection. Properly validated models (*i.e.*, those most consistent with the experimental data) are needed to accurately predict measured biomarkers of exposure and biomarkers of effect.

The sequence of events between exposure and response must be linked so that BBDR models can provide mechanistic insights on the origin of biological changes that occur at the cellular and molecular levels. These models can help identify biomarkers that are appropriate measures of exposure, effect, and susceptibility. Validated BBDR models can provide a sound scientific basis for extrapolating dose-response relationships across species and outside the range of experimental observation and thus reduce uncertainties in estimating human risk.

CROSS-SPECIES EXTRAPOLATION

Species-specific information at the cellular and molecular levels is critical for developing models that can be used to quantify relationships between time-dependent target tissue dose and tissue response as a function of exposure to hazardous agents. BBDR models combine toxicokinetic data on the absorption, distribution, metabolism, and elimination of agents at different levels of exposure with mechanistic data of time-dependent tissue response (*e.g.*, mutagenicity, altered gene expression). Species-specific mechanistic data, including parameters that are measurable in humans, are critical for developing these models. Experimental data are needed to estimate relevant parameter values (*e.g.*, tissue partition coefficients, enzymatic activities, binding constants) and to resolve uncertainties in the accuracy of parameter estimates, interdependence of parameters, validity of scaling methods, variability of parameters among individuals, and effects of co-exposure to other agents that may alter any of the critical biological processes. These models should evaluate similarities and differences in animal and human response as a function of the time-dependent tissue dose, whether the correct dose metric(s) have been specified for extrapolations, and whether responses in animals reflect the range of responses that might occur in exposed workers.

A major limitation to predicting the susceptibility or resistance of human neonates to chemical exposure is a lack of similar information about the development of xenobiotic metabolizing enzymes in rodent models of human toxicity. Differences in the extent of expression of xenobiotic metabolizing enzymes between rodents and humans at a given period of development complicates the interpretation of neonatal chemical exposure studies where rodents are used as models for humans. Thus, a systematic characterization of the ontogenetic development of key xenobiotic biotransformation enzymes and repair enzymes is needed in common laboratory animal models of developmental and reproductive toxicity (*e.g.*, the rat and rabbit) compared with that of humans. Such an analysis needs to be carried out using protein expression methods (including functional analyses) rather than just through mRNA expression. The objective of this work should be a map showing the degree of expression of key isoenzymes of xenobiotic metabolism over time in laboratory animal models and in humans.

DOSE RATE EFFECTS

In many situations, human risk assessment relies on toxicity data from studies conducted in laboratory animals under standard testing protocols. Compounds are administered at constant levels over regular intervals (*e.g.*, daily 6-hour inhalation

exposure) for defined periods of time (*e.g.*, 13 weeks; 2 years). On the other hand, human exposures rarely conform to these prescriptive dosing regimes. As a matter of practical consequence, a number of default assumptions with respect to dose rate and exposure duration have become implemented in risk assessment. Doses averaged over a work shift (in most occupational scenarios) or even a lifetime (in cancer risk assessments) are generally assumed to result in equivalent risk regardless of exposure pattern. Adverse response is often assumed to be linearly related to the product of exposure level times duration (Haber's Law). For example, 1 hour exposure to 80 ppm is equivalent to 8 hours exposure to 10 ppm (Andersen *et al.* 1987). For some endpoints (*e.g.*, irritation; some developmental effects), it is commonly assumed that exposure level dominates and duration has almost no influence on risk. Most of these default assumptions have not been rigorously supported by scientific research (Jarabek 1995). Recent studies by Weller et al. (1999) indicate that the developmental effects of ethylene oxide exposure depend on both exposure level and duration, but do not conform exactly to Haber's law. Similarly, some of the carcinogenic effects of 1,3-butadiene are more dependent on exposure level than exposure duration (Melnick *et al.* 1990)

Recent improvement in our understanding of the underlying determinants of toxicity and the ability to make quantitative predictions of tissue dosimetry should facilitate more focused research in the area of dose-rate effects. PBPK models are now able to relate the time course of a wide variety of internal dose metrics from a vast number of external exposure level and duration combinations. Time- and concentration-dependent processes such as metabolism must be accounted for, so that these models will be useful in selecting experimental conditions and interpreting results. The critical biochemical determinants of dose-rate effects (*e.g.*, interaction with molecular components, repair of cellular damage) must be incorporated into the framework of risk assessment methods. Finally, early cellular biomarkers of tissue dose and toxicity are needed for investigations of the temporal relationships at lower and more relevant exposure levels. These advances are needed to permit the development of more scientifically sound approaches in accounting for exposure pattern and duration in estimations of human risk.

TOXICOLOGICAL RESPONSE IN THE LOW-DOSE REGION

Quantitative risk assessments typically involve establishing a dose-response relationship; however, it is common that the exposures of interest for environmental risk assessment purposes are below the region where a response may be observed in experimental studies. For occupational chemicals, experimental studies at times include exposures in the range that has been encountered by workers. Toxicological investigations have traditionally required the observation of overt, quantifiable response in a relatively small samples of animals. This necessity is commonly addressed by using high doses in toxicological studies, compared to the typical region of interest for humans. The term "low dose region" refers to the range of exposures encountered by humans. For industrial chemicals, workers may be exposed to levels that are several orders of magnitude higher than those found in the general environment. High doses that cause generalized toxicity may lead to altered patterns of metabolism and elimination,

compared with those that prevail at lower doses. Mechanistic studies that are conducted only at these high doses may be misleading relative to mechanisms operating at lower doses. In these cases, extrapolation of the dose-response relationship to the low-dose region below the range of experimental data may be affected by the mechanism underlying the toxicity. Well conducted toxicity and mechanistic studies include multiple exposure concentrations that extend into the region where toxicity does not alter metabolism or elimination. The risk estimates derived from high-dose extrapolations depend critically on the estimated shape of the dose-response curve in the low-dose region. Greater understanding is needed of the shapes of typical dose-response relationships for cancer and non-cancer endpoints. This will lead to reduced uncertainty in quantitative risk assessment, and an increased confidence in the resulting risk estimates. Biomarker studies focusing on the mechanistic events that ultimately lead to an overt toxicological response hold the promise of extending the range of observable response into the low-dose range, which is more relevant to human exposures. Mechanistic biomarker studies are needed to better distinguish between linear and nonlinear responses. The ultimate goal of these studies should be to provide appropriate data for low- dose extrapolations, for both cancer and non-cancer endpoints.

USE OF CONTINUOUS DATA FROM TOXICOLOGICAL RESPONSES

Although considerable research has been reported concerning the use of quantal data in dose- response modeling, far less progress has been reported on the use of continuous data (Gaylor *et al.* 1998). Continuous data are often generated in the case of noncancer endpoint studies including those of reproductive toxicity, immunotoxicity, and neurotoxicity. Useful endpoints including body weight, enzyme activities, protein and neurotransmitter concentrations, cell counts, and neuronal cell death are usually reported as continuous data. Although continuous data can be converted to quantal values in some instances, substantial precision may be lost during this process (Gaylor 1996). Therefore, procedures must be established for using continuous data in dose-response assessment.

The most controversial aspect of the using continuous data for dose-response assessments is determining the "cut-off" value for defining an adverse effect. Defining this adverse level of change from controls is a critical decision and should be grounded on sound biological and toxicological principles. An ideal method should be based on the available data, apply to most continuous data sets, and minimize arbitrary decisions. Several approaches currently available include "amount of change" considered to be adverse by experts, use of an historically-based cut off for a particular continuous data endpoint, or amount of change in the experimental mean value based on the mean and standard deviation of the control data set. Data can then be modeled as continuous data, or be converted to quantal values. Although these and other approaches have been investigated to a limited extent (Gaylor and Slikker 1990; Crump 1995; Glowa and MacPhail 1995; Kavlock *et al.* 1995; Kodell *et al.* 1995; Slikker *et al.* 1996, 1998; Bosch *et al.* 1996; Chen *et al.* 1996; Gaylor *et al.* 1998, Haber *et al.* 1998), a systematic comparison of these methods is needed to develop a valid approach for using continuous data in risk assessment.

DEVELOPMENT OF TOXICOLOGICAL RESPONSE DATA FROM ACUTE AND SHORT-TERM EXPOSURES

Current testing approaches for acute and short-term toxicity tend to be limited or nonexistent. Often, the only acute data available are from studies that are designed to determine an LD_{50} or some form of severe toxicity and done for the purpose of dose-setting for longer-term (*e.g.*, 2-week or 90 day) studies. Some data from other studies are available and are used to derive an acute reference value; *e.g.*, clinical observations in the first few days of the subchronic study may be helpful in setting standards. Also, developmental toxicity data are often used for setting acute and short-term reference values even though the exposure periods may be as long as 10 days to several weeks. This is because it is presumed that most, if not all, developmental effects are possible to induce with single exposures. However, no acute or short-term data currently are developed on the aging population. Obviously, having pharmacokinetic information and understanding the mechanism of action of the effects induced would provide more information about whether they are appropriate for acute or short-term standard setting. Thus, testing protocols are needed that can be used for setting no-observable-adverse-effect levels (NOAELs) or benchmark doses (BMDs) for acute and short-term exposures, and for determining how to use data from other studies (*e.g.*, developmental toxicity, data in the aging population, other organ studies, and longer-term studies) in addition to appropriate adult toxicity studies. In addition, useful mechanistic and pharmacokinetic data are needed to aid in understanding the best approach for testing as well as using these data in risk assessment.

EXPOSURE TO COMPLEX MIXTURES AND MULTIPLE EXPOSURE ROUTES

Most toxicological testing conducted in experimental animals relies on administration of a single compound by a single route. On the other hand, humans are often exposed to mixtures of chemicals through multiple routes. In many occupational and environmental situations, it is increasingly recognized that risk of illness or injury may be the result of combined inhalation and dermal exposure to the same chemical source. To complicate matters, the chemical source may actually be a complex mixture of several substances, all of which may contribute to the risk. Exposure to chemical mixtures may also cause chemical interactions that could either potentiate or inhibit the expression of adverse response. Recent laws, such as the Food Quality Protection Act of 1996, contain provisions that require risk assessment to address aggregate exposures from multiple routes and cumulative risk from exposure to multiple chemicals with a common mode of action. New toxicological test protocols and approaches are needed to generate data on exposures to complex mixtures and multiple routes of exposure and to integrate that information into risk assessments. If appropriately constructed and validated with experimental data, BBDR models can be used to estimate the amount of internal dose (*e.g.*, blood or tissue level) from multiple routes (*e.g.*, inhalation and dermal contact) or predict interactions at molecular targets (*e.g.*, receptor binding) from exposure to two or more compounds.

UNCERTAINTY FACTORS

Default uncertainty factors of tenfold have been used traditionally for extrapolation from animals to humans, and to account for the variability among humans including sensitive sub- populations. Human health risk assessment can be improved by improving the choice of uncertainty factors and moving away from defaults, when supported by scientific evidence. A first step away from defaults is the use of categorical defaults based on characteristics of the substance or species differences. Uncertainty factors based on categorical defaults are used in the case of animal to human extrapolation for reference concentrations when dosimetric adjustments are done (USEPA 1998; Jarabek 1995), or in the use of surface area and metabolism adjustment for oral dosing. Renwick and colleagues (Renwick 1991; Renwick and Lazarus 1998) expanded the categorical defaults into a data- derived approach, in which the interspecies and intraspecies uncertainty factors are broken into toxicokinetic and toxicodynamic components, based on relative contributions of these components for a number of chemicals. The data-derived adjustment factor approach is being enhanced to make further use of the data, and allow the incorporation of chemical-specific data without requiring the detailed level of toxicokinetic information required to build a PBPK model. In addition, data-derived factors can address intra-species and inter-species toxicodynamic variability differences, but these data are more difficult to develop. Together, this hierarchy of approaches using increasing amounts of chemical-specific information allows the replacement of defaults with chemical- and species-specific information to improve the accuracy of the assessment. Research into a number of issues is necessary before factors based on chemical-specific data can be broadly used. For example, criteria need to be developed on how to evaluate whether the critical determining factor has been identified. Similarly, it will be necessary to clarify how information about human variability is used in kinetic models. Evaluation of PBPK models for chemicals acting via selected modes of action are needed to elucidate whether the distribution of certain parameters adequately describes human variability.

TOXICOGENOMICS

Genomics and proteomics have been widely hailed as fundamental technological breakthroughs in the evaluation of both biological response and biological susceptibility (Lovett 2000; Waring and Urlich 2000). The toxicological application of this technology is referred to as 'toxicogenomics.' Strengths of this methodology are the speed of screening a large number of genes and their responses to exposures, and the potential linking of a response to its underlying mechanism. This technology has potential usefulness for risk assessment, but may only represent the first step in a process that currently must include epidemiology and animal toxicology evaluations. Linking toxicogenomics to disease outcomes is needed before it can be routinely used as a risk assessment tool.

Of particular importance is the increasing availability of information about expression patterns of tens of thousands of genes, the link between them and protein production, and the translation to eventual adverse outcome. Availability of such information requires redefining biological responses to toxicants. Such techniques offer the potential to follow biological responses with time as occupational

diseases progress. They may offer new hope in identifying early biomarkers of toxicity as well as better assessments for cross-species extrapolation of data on biomarkers of effect and susceptibility. To effectively use such information, biomarker research needs to be expanded to enable such data to be put into effective dose-response and temporal contexts. In addition, guidelines are needed for collecting and interpreting toxicogenomic information for human health risk assessments. In particular, guidelines are needed to establish criteria for acceptable levels of sensitivity, specificity, accuracy, and predictiveness for gene expression as biomarkers of disease. Research is also needed to ensure that information obtained by these technologies is highly quantitative, include evaluations of time-dependent changes consequent to specific exposures, and adequately account for the effects of mixed exposures.

With advances in genomics and proteomics, identifying the complex gene environment interaction has become increasingly possible. Genetic testing, including all the elements of gene expression to protein production, promises a possible future presymptomatic determination. Current uncertainties regarding interpretation of the results from testing raise new risk management problems. Several complex ethical, legal, and social issues (though not discussed here) will arise with the advent of this new information. Therefore, research is needed regarding the most effective use of this genetic information and appropriate management strategies must be established (Fasouliotis and Schenker 2000).

CONCLUSIONS

In the coming decade, the application of experimental data to chemical hazard identification and characterization will require risk assessors to simultaneously address toxicological issues on three fronts. First, long standing and in many cases unresolved issues need to be addressed to improve traditional toxicological testing (*e.g.*, addressing exposures to complex mixtures and accounting for multiple routes of exposure) for expanded use in risk assessment and setting of regulatory standards. Second, new types of data including biomarkers of effect and susceptibility with corresponding data in both animals and humans are needed for improved species extrapolations and dose-response assessments. Lastly, toxicology will need to develop methods to properly use data from new developments in genomics and proteomics. The enormous quantities of data expected from these high through put technologies may require a revolution in the way data can be used in risk assessment for protecting public health. Priority issues that need to be addressed on these three fronts include inter- and intra-human variability and susceptibility with special emphasis on toxicological risks through all life stages (conception though senescence). To accomplish this, improved extrapolation is needed of experimental data to environmental and occupational human exposure situations. An essential component of this will be the linking of exposures to toxicological response, including exposure-rate and dose-response relationships. The development of biologically based dose-response models offers a mechanism-based approach to summarize all available data, identify data gaps, extrapolate dose- response relationships across species and outside the range of experimental observation, and account for factors influencing inter-individual differences in susceptibility.

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