

Workshop Report: State-of-the-Science for the Determination and Application of Dose-Response Relationships in Microbial Risk Assessment



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State-of-the-Science for
the Determination and Application
of Dose-Response Relationships in
Microbial Risk Assessment**

APRIL 21 - 23, 2009

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PREVENTION
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Disclaimer

This report was prepared as a summary of the presentations and discussions held at the U.S. Environmental Protection Agency / Centers for Disease Control and Prevention Workshop, State-of-the-Science for the Determination and Application of Dose-Response Relationships in Microbial Risk Assessment (April 21-23, 2009). This report captures the main points and highlights of the meeting; it is not a complete record of all detailed discussions, nor does it embellish, interpret, or enlarge upon matters that were incomplete or unclear.

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Acronyms and Abbreviations

AIC	Akaike's information criteria
BMD	benchmark dose
BMDL	benchmark dose 95% lower bound confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
CatReg	U.S. Environmental Protection Agency's set of categorical regression models also known as CatReg 2009 R Version
CDC	Centers for Disease Control and Prevention
CFSAN	Center for Food Safety and Applied Nutrition
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration
HACCP	Hazard Analysis and Critical Control Point
HIV	human immunodeficiency virus
ID50	dose that infected 50% of the test population
IgG	immunoglobulin G
IRAC	Interagency Risk Assessment Consortium
IRIS	Integrated Risk Information System
LD50	dose that caused death in 50% of the test population
LOAEL	lowest observable (or observed) adverse effect level
LOEL	lowest observable (or observed) effect level
LOTTEL	lowest observable (or observed) tolerable effect level
MRA	microbial risk assessment
NOAEL	no observable (or observed) adverse effect level
PA	Bacillus anthracis protective antigen
PBBK	physiologically-based biokinetic
PBPK	physiologically-based pharmacokinetic
POD	point of departure
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases
USDA	U.S. Department of Agriculture

Foreword

Following the terrorist events of 2001, the U.S. Environmental Protection Agency's (EPA) mission was expanded to account for critical needs related to homeland security. Presidential Directives identified EPA as the primary federal agency responsible for the country's water supplies and for decontamination following a chemical, biological, and/or radiological attack. To provide scientific and technical support to help EPA meet this expanded role, EPA's National Homeland Security Research Center (NHSRC) was established. The NHSRC research program is focused on conducting research and delivering products that improve the capability of the Agency to carry out its homeland security responsibilities.

As a part of its long term goals, one measure NHSRC has been charged with is delivery of reports and databases with information on the health effects of contaminants by 2012. Reliable dose-response data are critical to assessing the human health risks from exposure to microorganisms originating from intentional and unintentional releases resulting in contamination of buildings, drinking water systems, outdoor areas, or food. However, dose-response data for biological threat agents in the low-dose range are very limited. To bridge this critical data gap, advanced methods, animal studies, and other approaches are required to generate credible low-dose data to support the development of acceptable, scientifically-defensible response and remediation actions.

The April 2009, *State-of-the-Science for the Determination and Application of Dose-Response Relationships in Microbial Risk Assessment* workshop was held to discuss this and other data gaps in dose-response relationships in microbial risk assessment (MRA). This effort brought together many organizations across the country, including EPA's program offices, federal government agencies and laboratories, academia, and the private sector. Participants of the conference shared knowledge, explored differing opinions, and expanded overall understanding in MRA dose-response relationships.

This report represents a summary of the presentations and discussions during the workshop. We value your comments as we move one step closer to achieving our homeland security mission and our overall mission of protecting human health and the environment.

Cynthia Sonich-Mullin,
Acting Director National Homeland Security Research Center

Executive Summary

Both the U.S. Environmental Protection Agency (EPA) and Centers for Disease Control and Prevention (CDC) are tasked with preventing and mitigating risks presented by exposure to biological agents. These federal agencies employ microbial risk assessment (MRA) to inform the risk management decision making and risk communication processes through credible scientific data analyses. The practice of MRA has just recently expanded and, unlike the more formalized chemical and radiological risk assessment processes, is not as widely accepted or standardized. Therefore, various agencies and organizations have individually determined their own approaches, methods, and applications for conducting MRA to fulfill the agencies' respective missions.

EPA recognized these inconsistencies and the need to provide a forum to present and discuss various MRA methods and approaches employed by different organizations. Therefore, EPA initiated the first annual MRA Conference which was held in April 2008 in Rockville, MD. The conference had over 150 participants and included presentations on the mission-directed applications of MRA by scientists representing multiple federal agencies. Technical programs focused on current data needs and research advances in MRA hazard identification/characterization, exposure assessment, dose-response, risk characterization, and risk perception and communication. This innovative meeting allowed microbial risk assessors to showcase their research and collaborate with other scientists, risk managers, and stakeholders from academia, private-sector organizations, and federal agencies.

The success of and overwhelming participation in the first MRA Conference prompted a second annual EPA MRA Dose-Response Workshop in collaboration with the CDC, which was held 21-23 April 2009, in Atlanta, GA. As follow-on to the broader MRA presentations and discussions that occurred during at the first MRA Conference, this second workshop focused specifically on the dose-response relationships in MRA. The dose-response estimate describes the relationship between the exposure dose of a biological agent and the probability of adverse health effects. Reliable dose-response data are critical to assessing the risks from exposure; however, applicable and credible dose-response data for many biological agents are very limited.

This report developed from the 2009 workshop, *State-of-the-Science for the Determination and Application*

of Dose-Response Relationships in Microbial Risk Assessment, summarizes and highlights the presentations and discussions convened during the two and a half days. The primary goal of the conference was to share knowledge, explore differing opinions, and expand overall understanding in MRA dose-response relationships. Sixty-two workshop participants/subject matter experts represented federal government agencies and laboratories, academia, and the private sector. Dr. Cynthia Chappell, from the University of Texas School of Public Health, served as the keynote speaker. The remaining conference agenda consisted of 19 speaker presentations organized into five sessions:

- “Federal Mission Needs for Dose-Response”
- “Dose-Response Extrapolations”
- “Physiological-Based Modeling”
- “Dose-Response Methods Comparisons”
- “Dose-Response Applications for Vaccines and Therapeutics”

Following each presentation session, there were lengthy participant and presenter discussion periods. The technical content of this Report is based entirely on presentation information and discussions held at the workshop.

The objectives of the Dose-Response Workshop were to:

- Address the technical and scientific issues/challenges in MRA dose-response
- Discuss how to bridge critical data gaps using advanced methods to generate microbial dose-response data
- Examine novel approaches for the application of MRA dose-response data to predict human consequences
- Share knowledge, improve understanding, and identify data gaps for future research planning through strong participation by subject matter experts

Because of the diversity of attendees' disciplines, the different inputs and decision making required to support each organization's mission, and the limited timeframe, our aim was not to have participants arrive at a consensus on the best MRA dose-response approaches, methods, and data. Instead, the primary goal of the conference was to share knowledge, explore differing

opinions, and expand overall understanding in MRA dose-response relationships. The following are brief summaries of some notable discussion highlights:

- The key to the dose-response assessment is to determine what can be done to make the models useful and to recognize that the models do not need to be perfect as long as the uncertainties encountered in modeling projections are recognized and adjusted for. Multiple models will most likely be necessary to meet the challenges and required decisions. The critical focus for model selection is *who is making what decision and why*; the model should be built and utilized to inform the decision.
- The model needs to have the “right” complexity; very often there are too many parameters in the models. There must be a deliberate effort to choose the appropriate number of parameters in the dose-response model while avoiding confronting the issue of forcing the data to fit.
- MRA requires evaluations that indicate the adequacy of a dose-response model for the particular assessment being performed. This, in turn, requires clarification of assumptions being made regarding the processes involved in generating the data and determining outcomes. A model where these assumptions are obscure is insufficiently mechanistic.
- The key processes or steps of the microbial infection cycle (invasion, infection, illness) each have the potential for a dose-response threshold. Within each of these steps, there are potential barriers that can influence the threshold dose required to reach the next step. It remains difficult to discriminate between these key processes or steps and to identify and isolate the appropriate endpoints of invasion, infection, and/or illness caused by a single pathogen.
- Completely separating the exposure assumptions from dose-response modeling is difficult. A thorough understanding of the role of the environment in exposure is necessary to better define the contexts in which a biological agent exhibits pathogenicity and therefore the dose-response relationship.
- Pooling various dose-response data could allow more information to be gathered to enable stronger inferences as long as the differences in data sets can accurately be reflected and adjusted for.
- One of the greatest challenges with microbial dose-response modeling is the very limited availability of human dose-response data. As a result, the majority of dose-response estimates rise from experimental animal studies. To more accurately decrease the uncertainty arising from the animal-to-human extrapolations of dose-response data, microbial risk assessors can utilize species-specific physiological-based models.
- Another challenge with modeling microbial data from dose-response studies is that in most historical studies extremely high doses were administered to achieve effects, and therefore, the data require extrapolation from high-to-low doses to predict potential human responses at low doses. There can be large orders of magnitude differences in dose-response curve estimates in the resulting low dose extrapolations depending on the dose-response model utilized and the type and amount of data being modeled.
- Most dose-response models and data assume a homogenous human population and generally do not account for disease impact on sensitive subpopulations. Outbreak data can be particularly helpful in comparing the responses of healthy populations with potentially sensitive subpopulation (e.g., children, elderly).
- It is best to combine the risk communication process with the risk assessment/risk management processes early in the planning and to do so often during the entire process. Dose-response modelers need to communicate the assumptions, and strengths and weaknesses of their models up front so that decision makers and risk managers can interpret and apply the models correctly.
- Developing standardized microbial risk assessment terminology would be very valuable and would facilitate successful collaborations across disciplines. Communicating methods and results between disciplines has been difficult at times as the various disciplines sometimes apply different terms to define the same approach or the same term to define different approaches.

Keynote Address

Microbial Risk Assessment Dose-Response Challenges

Cynthia Chappell, Ph.D.

University of Texas School of Public Health

The keynote address, presented by Dr. Cynthia Chappell, focused on the advantages and challenges of conducting human dose-response studies. Dr. Chappell pointed out that one of the greatest challenges in assessing human health risk of pathogens is the lack of dose-response data. For most pathogens, data are absent or limited to studies conducted with surrogates and/or with laboratory animals that may not mimic human disease. Additionally, dose-response studies are generally conducted at high doses to ensure disease effects will be observed; such high doses may not be representative of doses relevant to human exposures.

Dr. Chappell presented an overview of the feasibility and ethics associated with collecting and analyzing dose-response data from human studies. Her presentation focused largely on her experiences with human exposure studies to different *Cryptosporidium* species and dosages. In this case, the dosing studies done in healthy adults were appropriate since the infection is self-limiting. The *Cryptosporidium* challenge studies took place over 11 years (1993-2004) and involved 186 individuals. The objectives of the studies were to describe the natural history of infection, identify the dose that infected 50% of test population (ID_{50}), calculate illness attack rates, and evaluate immune responses to infection.

The logistics of conducting human dosing studies require considerable planning to address the ethical and safety issues associated with infecting healthy people with a pathogen, to ensure that volunteers are mentally and physically qualified to participate in the study and are available when the pretested inoculum is ready for use. Unexpected and adverse events require special attention during such studies and should be part of the planning process.

Dr. Chappell summarized that, along with more carefully-collected dose-response data, there is a critical need for increasing knowledge regarding virulence factors and for better data integration from multiple animal and epidemiological outbreak studies.

Presentation Sessions

Session 1: Federal Mission Needs for Microbial Risk Assessment

Representatives from the U.S. Environmental Protection Agency (EPA), Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), and the U.S. Department of Agriculture (USDA) presented the mission requirements for microbial risk assessment (MRA). Specifically, each presenter was asked to address: “How is microbial dose-response information utilized in your agency’s decision making?”

Microbial Risk Assessment for the Development of Cleanup Goals

Tonya Nichols, Ph.D.
EPA

EPA conducts risk assessment of environmental contaminants to inform risk management decisions. To this end, detection capability must be in place to determine that a release has occurred, containment and mitigation protocols must be accessible, and remediation goals and strategies must be assessed and evaluated.

The overall role of the risk assessment is to determine how much of the contaminant would lead to an adverse health effect. This information guides clearance decisions, identifies how sensitive our analytical detection capabilities must be to determine the presence or absence of harmful concentrations of a contaminant, and defines “how clean is clean” to determine if the decontamination effort has been successful. EPA has a history of developing chemical target concentrations and using associated information to support decision making. Target concentrations that are currently used by EPA’s regulatory programs include *preliminary remediation goals* in the Superfund program, *health advisories* and *maximum contaminant levels* in the Office of Water, and *reference concentrations* and *inhalation unit risk* in the Office of Air Quality Planning and Standards.

Risk-based goals are a function of the target risk, intake, and pathogenicity. The risk-based goal can be used to derive target concentrations that can be compared with sampling results. To develop target concentrations for pathogens, the approach must be determined and the minimum data requirements should be identified (Figure 1). To date, the minimum data set required for setting clean-up goals for biological agents has not been determined. Existing guidelines, such as the “Animal Rule” (21 CFR 601) and Minimum Data Requirements for Registering a Chemical Pesticide (40 CFR 158) may

provide insights. Considerations for determining the minimum data set include: extrapolation of data obtained from surrogates to pathogens, defining differences between exposure routes, use of high dose data when low dose data would be more relevant, applicability of animal models, and determination of associated correlates of disease.

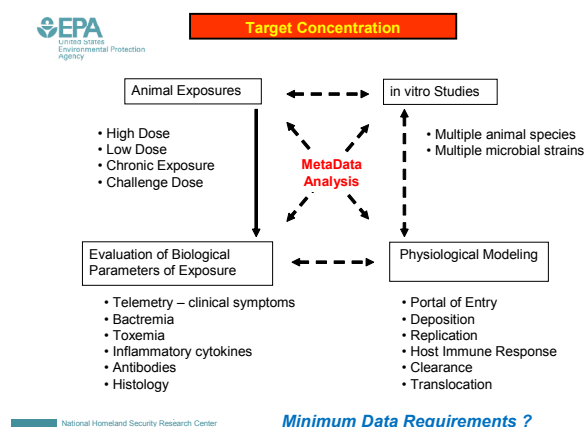


Figure 1. The determination of risk-based target concentration is complicated by the number of potential approaches and the assessment of minimum data requirements.

Because there is no consensus on the minimum data required for deriving a cleanup goal for biological contaminants, Dr. Nichols provided the following questions to stimulate discussions on identifying the research needed to derive a cleanup goal:

1. How do we approach a no observable adverse effect level (NOAEL) / lowest observable adverse effect level (LOAEL)/ lowest observable tolerable effect level (LOTEL) for exposure to microorganisms?
2. What dose-response models do we use and why?
3. How do we design *in vivo* and *in vitro* studies to better inform physiological modeling?
4. How do we extrapolate animal study data to humans?
5. How do we account for uncertainty and variability?

Infection Transmission, Infection Control

Michael Bell, M.D.
CDC

From an infection control perspective, disease management conducted in health care facilities focuses on stopping transmission from person to person, or from person to environment to person. With regard to emerging diseases, there seems to be a consistent pattern: 1) disturbance of or intrusion into ecosystems, 2) primary entrance into human host, 3) secondary spread among humans, and 4) potential amplification in health care facilities.

Disease transmission requires a number of steps to occur (Figure 2). Infection control to minimize the transmission of pathogens can essentially lead to zero exposure. Survival in the environment during transit can be affected by a number of environmental factors (temperature and humidity), droplet size, and composition. Transmission-based precautions for droplet and airborne transmissions should address infectivity relative to the time/distance of travel and the predominant transmission mode. For example, the strict 5 μ M cutoff value for aerosol inhalability originated from studies specifically related to tuberculosis and does not represent the upper size limit for inhalability for other pathogens. It also should not be assumed that all inhalation pathogens must reach the terminal alveolar region to initiate infection.

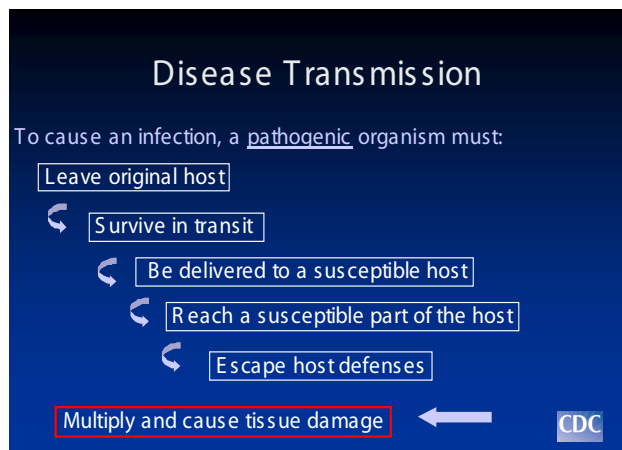


Figure 2. Necessary steps for disease transmission.

Systematic assessments of infectivity should consider questions about assessing pathogens separately by their features (e.g., viral envelopes), using representative organism versus specific organism, and using time as a surrogate for distance. CDC's current research agenda includes aerobiology and improvement of protective equipment for health care. Aerobiology considers organism-specific measurements, environmental variables, and substrate variables.

Mission Needs for Dose-Response at FDA-CFSAN's [Center for Food Safety and Applied Nutrition] Microbial Risk Assessment Program

David Oryang, M.S.
FDA-CFSAN

FDA has a long history (since 1906) of managing risks, conducting safety assessments and performing risk assessments for food additives, chemicals, and microorganisms. CFSAN's mission is to promote and protect public health by ensuring that the U.S. food supply is safe, sanitary, wholesome, and honestly labeled, and that cosmetic products are safe and properly labeled.

U.S. food safety is challenged by constant changes in the food system including: 1) significant increases in the volume, variety, and complexity of imported foods, 2) shifting demographics, 3) more convenience foods being eaten year round, and 4) new foodborne pathogens with relatively little available data. Each day, industry and government agencies must make decisions about the safety of foods and food products. The public health and economic consequences of "bad" decisions can be substantial; *not deciding* is not an option. There has been tremendous effort in the food safety community to make consistent and transparent decisions that are informed by science and risk.

Growing responsibilities and new challenges require federal regulatory agencies to develop new tools and approaches. CFSAN is moving toward a more risk analysis based approach, developing and using efficient means to collect, organize, review and share information used in regulatory decisions, and prioritizing activities in view of limited resources. Risk assessment is one of three components of the risk analysis triad: assessment, management, and communication. It is a process to describe what we know and how certain we are of what we know, and to answer four key questions: a) What can go wrong?, b) How likely is it to occur?, c) What are the consequences?, and d) What factors can influence it?

Risk assessment is a tool, used by CFSAN to:

- Support food safety decision-making – particularly when decisions must be made under uncertainty and all of the desired data are not available.
- Support decision making for import policies, control strategies, inspection programs, and safety tolerance levels.
- Assess the effectiveness of interventions by evaluating control measures, proposed standards, and the contribution of compliance to risk management.

- Inform communication and outreach strategies by identifying subpopulations that are at high risk, assessing uncertainty and variability, and presenting a comparison of alternatives.
- Assist food safety management with decisions by providing advice on where to look for hazards, by setting priorities and allocating resources, and identifying risk drivers along the “farm-to-fork” continuum.

One of CFSAN’s typical targets for risk management is microbial contaminants in food and food additives.

Looking forward, a significant microbial dose-response need for FDA is the identification and characterization of susceptible populations. A number of other FDA microbial dose-response related needs (presented in Figure 3) are identified. One recognized important need is the evaluation of growth models that would provide greater effectiveness in estimating exposure levels. A further need is to move the focus from acute (where it currently is) to transient and chronic effects (where there is currently little emphasis).

There is a need to increase accessibility to data, models and information, and to develop dose-response relations for new food-borne pathogens, by extrapolating data acquired in animal models to humans.

Key data needs are: a) descriptions of the variability in susceptibility; and b) variation in the infection to hospitalization ratio; within and between age groups and susceptible populations.

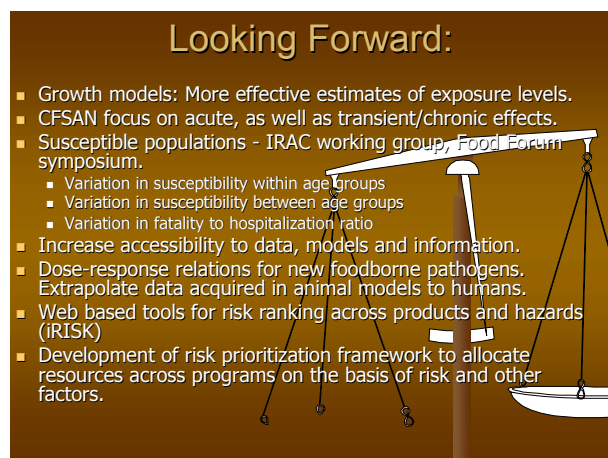


Figure 3. Microbial dose-response research needs and future directions of work as identified by the FDA.

(IRAC is the Interagency Risk Assessment Consortium)

CFSAN is developing a Web based tool (iRISK) to rank risks across products and hazards.

FDA’s ultimate goal is the development of a risk prioritization framework to allocate resources across

programs on the basis of public health risk and other factors.

To address microbial risk assessment issues, participants were challenged to learn from past experiences, develop new ways to address complex food safety issues, foster involvement of multi-disciplinary expertise, and actively participate in international activities.

Mission Needs at USDA

Janell Kause, MPH, MPP
USDA

USDA research is focused primarily on risk ranking and other parameters of relative risk. Dose-response analysis previously received minimal consideration as other risk assessment elements were further developed in the USDA; in particular, exposure analysis received significant attention. From a USDA perspective, exposure analysis evaluates foodborne exposure from the plant to table (i.e., “farm-to-fork”). Currently, attention is re-focusing on dose-response.

Data for dose-response are obtained from both animal and human studies. Challenges with animal data include limitations of scaling from animal to humans, conversion from mortality to morbidity, and the associated significant overall uncertainty. With regard to human studies, these have been conducted with healthy human populations, which may not be informative for susceptible populations. More often, there is a reliance on epidemiological data obtained during outbreaks of foodborne illness. Limitations from outbreak data include: 1) insufficient information on exposure level or the amount of pathogen consumed, 2) unknown pathogen sub-type or its associated virulence, 3) limited ability to recall food or food vehicles, 4) unspecific endpoints, and 5) the subjective determination of which outbreaks should be included in the epidemiological data. When comparisons between risk models and outbreaks are conducted, it can be shown that outbreak data and the modeled response data usually do not line up. There appears to be an underestimate of the dose necessary to induce health effects for some low dose exposures. The key issue is the lack of understanding of who is susceptible to what microbial hazard.

The inclusion of more specificity in the dose-response assessment will allow for a more refined focus on the hazard, better recall of contaminated food vehicles, and enhanced risk communication with those who are truly at risk. A better understanding of susceptible populations is needed; currently, the FDA uses age as the proxy for susceptible populations. An additional element that is necessary to advance the dose-response assessment is the ability to make decisions with increased certainty, especially for low-dose exposures.

Session 2: Dose-Response Extrapolations

Presenters were asked to address the following stimulus questions:

1. How is uncertainty and variability addressed in extrapolating dose-response data (e.g. extrapolating across host species, exposure levels, routes of exposure, durations of exposures, pathogen strains or species, endpoints, and/or sensitive populations)?
2. Is it appropriate to group studies, animal models or host species, and/or pathogen strains or species in dose-response modeling of multiple data sets?

Accounting for Uncertainty and Variability with Mechanistic Knowledge in Dose-Response Assessment

Margaret (Peg) Coleman, MS

Formerly affiliated with Syracuse Research Center

The short answer to both stimulus questions on extrapolation is:

- Be skeptical.
- Examine the body of evidence available on mode/mechanism of action and dose-dependencies for disease resistance and susceptibility.
- Pool only with scientific justification.
- Extrapolate using knowledge of the disease triangle and mode/mechanism of pathogenesis/virulence.

Examples presented to support the above answer featured an integrated dose-response methodology that linked empirical and mechanistic knowledge in mice and humans for anthrax, salmonellosis, and tularemia (See Appendix C for details of specific examples). Such methodology provides a more robust assessment of dose-response relationships by incorporating variability in all aspects of the disease triangle (host resistance and susceptibility, pathogen infectivity and virulence, and environmental influences on exposure and disease progression). Prototype physiologically-based biokinetic (PBBK) models for anthrax and tularemia, akin to physiologically-based pharmacokinetic (PBPK) models for chemicals, support scaling of external and internal doses to target tissues that are stronger predictors of outcome (resistance or susceptibility to disease) and disease severity. More integrated knowledge will better inform decisions about extrapolation and pooling. For many infectious diseases, the tissue tropisms and pathology differ by route and host, so to reduce

uncertainty in extrapolation, critical species-common effects must be identified.

PBBK modeling illuminates the black box of dose-response assessment and expands our limited ability to predict resistance and susceptibility to pathogens, and the likelihood and severity of human disease under conditions of susceptibility. Two groups independently developed mechanistic models for inhalation anthrax adapting existing methodology from chemical risk reported in the EPA Integrated Risk Information System (IRIS) to interrelate empirical and mechanistic models for respiratory system pathogens. A team of scientists at Syracuse Research Corporation prepared anthrax PBBK models for guinea pigs and primates, and subsequently extended that work to develop a prototype PBBK model for tularemia in primates and humans (see Lumpkin presentation).

Empirical models for non-human primate datasets are available for multiple endpoints and *Francisella tularensis* strains that can expand when linked with PBBK models using integrated dose-response assessment methodology. The empirical model for non-human primates, informed by the PBBK model, predicts an internal dose-response function. This predicted internal dose-response function then informs the human PBBK model to predict human internal and external dose-response models based upon the additional endpoints and strains observed in the non-human primates. In this case, existing human curves for infectivity from volunteer studies can be used to exercise the methodology in reverse i.e., predict non-human primate curves as an additional check on the validity of our approach.

The scientific basis of the current practices that focus on empirical modeling can be improved by accounting for mode/mechanism of action, particularly variability in aspects of the disease triangle. Some datasets, including salmonellosis and tularemia, offer both human and animals dose-response datasets that could prove useful for testing hypotheses and validating dose-response assessment methodology. Advancing prototype mechanistic models of disease in respiratory, gastrointestinal, and dermal systems would facilitate model-directed research that could further refine our models and expand our knowledge of low-dose behaviors for significant pathogens. Such knowledge is key to selection of 'safe' levels unlikely to cause disease (as per the statistical threshold demonstrated for *Salmonella pullorum* for humans) or severe disease (as per tularemia endpoints for primates).

Let the Data Speak

Chuck Haas, Ph.D.
Drexel University

Intrinsic maximum likelihood fitting techniques can be used to account for experimental variability; other mathematical techniques can be used to evaluate parametric uncertainty. Statistical tests can be conducted to assess the appropriateness of pooling. Tests have been conducted and decisions made to pool data between strains, species, hosts, and sensitive subpopulations for various published data sets. Pooling of data can be conducted if the data justify it based on statistical and biological rationale.

The dose metric should be an ingested or inhaled number but other dose metrics may also be appropriate. One current Drexel project is evaluating *in vivo* pathogen dynamics to assess whether body burden is an appropriate metric. It is also possible that body burden, area under the curve, or other measures may be appropriate.

The mechanistically derived dose-response models (exponential, beta-Poisson) have been found to be consistent with all data sets examined to date, including human data on: *Legionella pneumophila*, *Salmonella typhimurium*, *Giardia lamblia*, *E. coli* O157:H7, *Cryptosporidium parvum*, *B. anthracis* (Sverdlovsk), and severe acute respiratory syndrome (SARS). In many cases, it has been possible to validate use of such models against outbreak data.

NOAEL, LOAEL, and Dose-Response Curves: Lessons from Anthrax

Thomas Whalen, Ph.D.
Georgia State University

Extrapolation models (e.g., linear, logit, probit, log probit) have all been used for dose-response modeling of anthrax data. Many publications involve extrapolation from high dose animal studies (especially Jemski's unpublished data from Glassman, 1966) to assess potential adverse effects on humans arising from extremely low doses (e.g., nine spores infecting 2% in Meselson's analysis). However, close examination of the published historical accounts of actual human exposures do not support anthrax disease after exposure to such low dose levels. For example, Holty's review of diagnosed anthrax cases over 107 years only found 32 documented cases. Furthermore, the evidence indicates that the 1957 Manchester outbreak originated from egregiously contaminated goat hair; however, air monitoring after the outbreak had ended found hundreds of spores in the air at a time when no new cases were identified.

Brachman exposed macaques for 47 days to *B. anthracis*-contaminated air from a working South Carolina mill. Measured environmental concentrations were found to be highly variable (ranging from tens to a few hundred spore-containing particles inhaled by each macaque in a day). Under these varying conditions, a number of monkeys developed inhalational anthrax. Dr. Whalen and colleagues used Brachman's published data (1966) to estimate the number of spore-containing particles inhaled by humans working in the mill, based on human respiratory rates and an air cleaning system with approximately 90% efficiency. The result was an estimate that workers in this mill setting would likely have inhaled over 600 spore containing particles per day for 36% of the 47 days of Brachman's study. Extending this to the estimated number of worker-days in the 60 years from the beginning of the twentieth century mill ventilation in the United States to widespread vaccination of mill workers around 1960 yields approximately 15,000 unvaccinated worker days associated with doses greater than 600 spore containing particles inhaled per day in the mill – with fewer than ten cases of inhalational anthrax documented. Dr. Whalen proposed 600 spore containing particles per day or fewer as a potential NOAEL. Likewise, a LOAEL can be developed based on the assumption of approximately 18 million worker days across all mills with over 600 particles (during the period of 1900 to 1960), with at most nine cases of reported inhalational anthrax disease. It was noted that these exposures are likely to include spikes in anthrax spores in the air significantly greater than 600 spores inhaled per worker per day.

Furthermore, observational data seem to contradict the results obtained from low-dose extrapolation of experimental data as conducted by Meselson and many others. For example, millworkers' wives and children were likely receiving more than nine secondhand spores yet they were not experiencing anthrax-related illness. Likewise, workers themselves were likely experiencing workplace conditions with non-zero exposures to anthrax spores – yet were not exhibiting anthrax illness in the numbers anticipated based on the worker population pre-1965 prior to introduction of the vaccine.

Dr. Whalen asked the following questions: Which is better – the use of imperfect data based on thousands or even millions of human exposures at low doses or experimental laboratory data based on dozens of animals at high doses? The challenge today is how to integrate the two disparate results. Considerations of who will make the decision and how it will be used are important factors that may guide the integration process.

Impact of Animal Models

Mary Alice Smith, Ph.D.
University of Georgia

One of the known issues in the low dose region of the dose-response curve is the fact that the fitted models with relatively similar estimates in the middle dose regions may provide very different response estimates in the lower end of the curve (Figure 4). The current challenge is the determination of which curves may be correct. These modeled example data represent doses outside the range of the test that generated the original data and are outside the realm of typical animal studies given the low probabilities of occurrence.

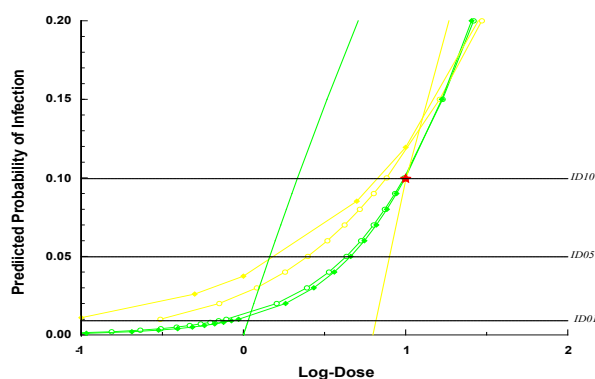


Figure 4. Comparison of multiple fitted models to show different extrapolation results in the low dose region of the curve.

One concept meriting consideration is the International Life Sciences Institute “Thresholds in the Dose-Response for Bacterial Pathogens Key Events” approach. Key events are those events along the pathway between intake and ultimate effect. This approach has been successfully used by EPA in the evaluation of thresholds in the dose-response analysis of chemical hazards and may have applicability for determination of thresholds in microbial hazards. Overall, dose-response relationships will reflect a summation of steps, and the process of understanding and modeling each step will lead to a superior modeling approach. It will allow for prediction of the variation in response due to human variability or microbial strain, and may allow for evaluation of the interaction between quorum sensing and the immune system. There is value in the use of an iterative approach: 1) develop animal models with the available data, 2) match human incidence data on dose-response to identify closest animal model dose-response relationship, then 3) consider mechanisms involved to further refine animal and model selections.

Session 3: Physiological-Based Modeling

Presenters were asked to address the following stimulus questions:

1. What overall assumptions are necessary for valuable physiological models to predict human consequences?
2. What is the minimum data set required (i.e., what level of detail needs to be modeled for acceptable human predictions (e.g., whole species models, organ-specific models, and/or cellular or toxin activity models)?)

Physiologically-Based Modeling

Sarah Taft, Ph.D.
EPA

Assessing the human dose-response relationships for microbial agents is often challenging as actual human data is very limited and, in most cases, non-existent. Therefore, to estimate these human dose-response relationships, especially with regard to biological threat agents, data must be extrapolated from experimental animal models of infection and disease. To date, however, there is no standard approach or consensus-based methodology for animal-to-human microbial data extrapolations.

For chemical risk assessments, the NOAEL observed in the animal model, is divided by some magnitude of an uncertainty number to account for the species differences and interspecies extrapolations from animal-to-human. EPA has a long history in conducting chemical risk assessments, so Dr. Taft raised the question, can an approach that is similar to that applied for chemical risk assessments be applied to microbial hazards where an uncertainty factor of some magnitude is used to account for the uncertainty in the animal-to-human microbial data extrapolation?

Dr. Taft went on further to ask the following questions: What can we learn from these approaches? How can we reduce the uncertainty in animal to human extrapolation? And, are these chemical dose-response analysis approaches appropriate for microbiological hazards?

Consideration of interspecies differences, as is done in chemical risk assessments, could reduce uncertainty in animal-to-human extrapolations for microbial agents. Interspecies differences could be broken down into kinetic and dynamic elements for physiologically-based modeling (Figure 5). Kinetic elements are physiological factors affecting the ability of the microorganism to reach the target tissues. These typically include

dosimetric concepts relating to a deep dose in the lung and other targets; examples of kinetic differences with regards to *B. anthracis* infections include bacteria clearance from alveoli, bacterial germination rate, lymph node bacterial dose, bacterial dose in circulation, bacterial toxin production, and bacterial replication. Dynamic elements are differences in the effects at the target tissue (i.e., the response); these include bacterial toxin activity and host inflammatory responses.



Dose-Response Assessment for the Development of Cleanup Goals

How can we decrease uncertainty in animal-to-human extrapolations for biothreat agents?

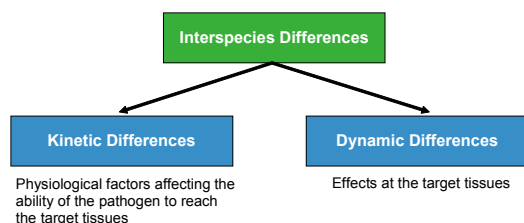


Figure 5. Considerations of interspecies differences that may reduce uncertainty in animal-to-human extrapolations in microbial dose-response assessment for the development of cleanup goals.

Physiological-Based Modeling in Microbial Risk Assessment

Jeff Gearhart, Ph.D.,
Wright Patterson Air Force Base

Dr. Gearhart prefaced his presentation with three key statements regarding physiologically-based biological modeling in MRA:

- Currently, physiologically-based biological models in MRA are primarily research tools – quantitative methods for hypothesis testing with experimental data.
- Physiologically-based biological models are NOT intended to replace other modeling approaches but are hopefully an adjunct to other modeling approaches.
- Physiologically-based biological models may not be required or necessary for all MRA applications.

The biggest assumption in physiologically-based biological modeling is that there is an understanding of the actual mechanism(s) of pathogenesis for the microorganism of interest. The host-pathogen interaction

must be understood to derive quantitative measures of this interaction. It is also imperative to understand the immunological processes i.e., similarities and differences between hosts and experimental animals, for both the animal host and the human, to conduct animal-to-human extrapolations. This understanding has been a challenge with the re-analysis of historical experimental animal data. Importantly, the assumptions should also depend on the questions being asked (e.g., the focus on death as an endpoint in past assessments limits the knowledge that can be gained from these data).

The main motive for the development of different physiologically-based biological models for various agents is to understand the actual mechanisms of pathogenesis. Physiologically-based biological model development starts with an overall mechanistic schematic of the infection process – route of microbial entrance into the host, initial microbe response, and subsequent host response. Quantitative laboratory measurements can be used for endpoints input into the model, and these endpoints can in turn also be used to evaluate model predictions. The quantitative measures of the host-pathogen interaction are the most critical physiologically-based biological model elements.

The largest drawback of the modeling approach is how “data hungry” the physiologically-based biological models are. Determining the minimum data required depends on how global the physiologically-based biological modeling approach is. There is considerable animal data available in the literature and from the lab, but the question becomes, can the data be utilized in and can it advance the physiologically-based biological model (e.g., incorporating *in vitro* data in a whole animal model)? Furthermore, the ultimate goal is for these models to predict the potential human consequences; therefore, the corresponding data and parameters first modeled for experimental animals must subsequently be coded for humans in the overall model.

Most of the existing studies in the historical literature for modeling anthrax use high dose exposure data. Reliance on high dose data confounds the identification of relevant mechanisms, particularly with respect to the relationship of time to dose. Physiologically-based biological model development for anthrax requires quantitative information on the following data elements: spore deposition in the alveoli, ingestion by the alveolar macrophage, germination and replication of vegetative bacteria, transport to the lymph nodes, defeat of the macrophages, and active *B. anthracis* replication producing bacteremia and toxemia.

Developing Mechanistic Models for Risk Assessment of Biothreat Agents

Michael Lumpkin, Ph.D., DABT
Syracuse Research Corporation

To address the first question of what assumptions are required for physiologically-based models, the ultimate application of the model must first be defined. There are three main uses of physiologically-based mechanistic models for dose-response analysis: 1) retrospective applications - these include extrapolation of an observed dose-response relationship in animals-to-human receptors and are commonly conducted for chemicals, 2) prognostic applications - these allow for the prediction of health outcomes after a biothreat incident, and 3) prospective applications - these allow for exploration of measurable forensic biomarkers and can also be used to back-extrapolate from outcomes to exposures or to inform identification of health outcomes from a given exposure.

The output of the physiologically-based mechanistic model is typically a computer simulation of events from exposure to disease. To produce defined dose-response empirical relationships, these models derive outputs from the knowledge of biology along with the understanding of the pathogen kinetics and dynamics. As modeling increases in biological detail, it is anticipated that uncertainty is reduced (Figure 6). However, the simple addition of more biology doesn't necessarily give you more certainty. The overall desire is that models are useful outside very narrow applications.

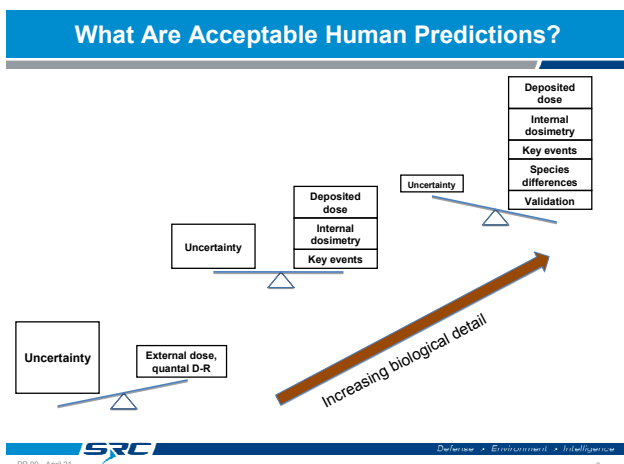


Figure 6. Uncertainty in physiologically-based mechanistic modeling may be reduced with the addition of increasing biological detail.

Minimal data requirements increase as modeling moves from empirical to the more physiologically-based mechanistic approach. As additional data elements are added with more mechanistic information, it is believed that the predictive capacity increases and movement

is allowed along the continuum of retrospective, prospective, and prognostic applications. This movement appears to be a generally linear process to initially gather and supplement the available data. However, Dr. Lumpkin raised the question, what happens when the data gap is at the initial step of the identified empirical relationship stage? To address this challenge, the best overall approach may be to develop models for multiple agents in concert and to share data parameters across agents. With such a cross-cutting approach, identifying one data element for one tract that is missing in another could be very beneficial and could greatly advance the development of microbial physiologically-based models.

Syracuse Research Center is developing physiologically-based models by using the general approach of moving from empirical to mechanistic. The following assumptions are utilized in their particle inhalation model: 1) generalizations about deposited doses capture relevant details of exposures, 2) *in vivo* pathogen growth rates change as a consequence of host-pathogen interactions, 3) the *in vivo* pathogen and toxin rates are biologically justified, 4) a critical species-common effect has been identified; and 5) an internal dose metric has been identified that is sensitive to the critical effect.

Session 4: Dose-Response Method Comparisons: Classical, Bayesian, Epidemiology, and Benchmark Dose Modeling

Presenters were asked to address the following stimulus questions:

1. Is the dose-response statistical method utilized empirical or mechanistic?
2. Is the method applicable for low-dose extrapolations?
3. Can the method accommodate data pooling and/or the use of correction factors?
4. How is the calculated dose-response relationship verified and validated?
5. How is model uncertainty adjusted for and communicated to risk managers?

Dose-Response Method Comparisons: Classical Studies: Quality assurance process and techniques for leveraging new and old data

Tim Bartrand, Ph.D.
Clancy Environmental Consultants

Mechanistic models are derived based on assumptions regarding the probability distribution of the dose and the probability of a single pathogen initiating an infection. If it is assumed that all pathogens have equal probability of initiating infection, the model is exponential (i.e., if the

probability is constant). If the probabilities of pathogens initiating an infection are beta-distributed, the model is a beta-binomial; the assumed host response may also be beta-binomially distributed.

Classical dose-response models are mechanistic as they are based on biologically plausible processes (e.g., dose or dose distribution is known and infection can be modeled as a single event or as a sequence of events). Low-dose linearity represents a finite probability that a single organism can initiate infection. However, models can be increasingly complex to incorporate considerations regarding competitive processes, survival or time to infection modeling, and fractional dose models.

The following quality assurance process has been used by Dr. Haas' lab at Drexel University in the development of classical dose-response models. First, the data are screened to evaluate the suitability of the data for use in dose-response modeling. Considerations for the data evaluation include knowledge of the pathogen strain, origin and features, exposure route definition, dose or environmental concentration, defined endpoint, animal description, and observation of intermediate response measured (not necessary when using lethality endpoint data). A test for trend (Cochran-Armitage) is also conducted; it should be noted that some data sets (e.g., Lassa virus aerosols) have a very flat curve where the trend is not visually obvious. The data determined suitable for dose-response modeling are fit with the classical models using a maximum likelihood method. Goodness of fit can be examined by a chi square test with fit assumed when the deviance is less than the chi square value based on the number of dose groups minus the number of parameters in the model. The best fit model has the lowest deviance. A bootstrap process can be used to generate confidence intervals for the communication of variability and uncertainty. The outputs of this analysis include 1.) distributions associated with parameter estimates and 2.) confidence intervals on the percentile response value.

With regard to pooling, a statistical test in combination with other biological considerations is used to determine the acceptability of pooling data. Biological considerations include the support of pathological findings for pooling and determination that no systematic differences exist between populations (e.g., inbred versus wild, prior exposure/immunity, age, diet.). An example was presented regarding determining the appropriateness of pooling two human and animal dose-response data sets for tularemia inhalation exposure. Curves with statistically valid fits were developed using the beta-Poisson model for the human data and exponential model for the monkey data. While the individual data sets could be fit to available models, the pooled data

could not. Pooling analysis can be used to determine whether responses of animal hosts come from the same distribution, or to compare results of multiple experiments.

Models and associated codes should be verified; model components have to be benchmarked, the models themselves have to be robust, and multiple users have to produce the same results when using them. Models should also be validated; multiple data sets, outbreak data, time-to-response data, or comparison of models for multiple routes can all be used to validate models. These validation data sets oftentimes have limitations which should be taken into account (e.g., limited dose data in outbreaks).

Dose-Response Comparisons: Bayesian Statistics

***Jade Mitchell-Blackwood, M.S.
Drexel University***

Bayesian analysis can reflect a broad array of analyses. Its essential concept is the application of Bayes' Theorem to learn from available previous observations. Parameters are defined as random variables, rather than the discrete values used in Classical dose-response models. Bayesian analysis can be applied to both empirical and mechanistic dose-response models. The use of exponential and Beta-poisson models for earlier work was described by Haas et al. as mechanistic. The rationale for terming these models as mechanistic is that they have been based on biological plausibility relating to random doses with a Poisson distribution in the dosing medium. It is assumed that there is a probability that one or more organisms may be ingested by the host and there is a probability of survival for a single organism once it is ingested, rather than quantitative assessment of individual organism survival rates. These models have also been termed "mechanistically-based empirical" because the parameters are determined empirically from curve-fitting response data.

Bayesian methods can be applicable for low-dose extrapolations if the model being fit can be used for low dose extrapolation. For example, the exponential and Beta-Poisson models are based on assumptions that, if true, would allow for low-dose extrapolations. However, obtaining adequate low dose data to validate these assumptions is a challenge.

Bayesian methods can allow for methods to accommodate data pooling and the use of correction factors. Hierarchical models can be used to perform meta-analysis without the need for the pooling assumptions required by the classical approach. It should be noted that Bayesian analysis is unique in its ability to handle hierarchical modeling relative to other dose-response statistical methods. Using data from Bartrand

et al. (2008), an inter-species variation example can show the potential for Bayesian analysis to use available data for a number of different species. In this example, it should be noted that Bayesian analysis allows for the use of the white rabbit data (with 100% lethality at all doses tested) which would not normally be useful for determination of dose-response analysis using other methods. Bayesian methods can use this data along with the data for the other host species to generate unique parameter distributions for each species individually and a generalized parameter distribution, based on the hierarchical model, for all species observed (for which observations are initially available) and unobserved (for which predictive distributions are required).

When using Bayesian analysis, the calculated dose-response relationship can be verified and validated through a number of different complementary approaches. As with other techniques, graphical plots of model and data are a good first step to evaluate fit. The Bayesian Information Criterion and Deviance Information Criterion can be used to score fit. The Bayesian Information Criterion is similar to the Akaike's information criterion (AIC) in that it is calculated as a log-likelihood with a penalty for the number of parameters. Models can be cross-validated if there are sufficient data that were not used to generate the model. Verification of the lack of impact of the assumed prior distribution on the resulting posterior distribution can be assessed by using both an informed and an uninformed prior distribution and comparing the results. To show that the results are unbiased, the resulting posterior distributions should be similar. Model uncertainty can also be evaluated and reported, using Bayesian analysis, by calculating credible intervals from the posterior parameter distributions.

Modes of Action in Low-Dose Extrapolation

Laurie Waisel, Ph.D.,
Concurrent Technologies Corporation

Modes of action are important to consider when conducting low dose extrapolations. A mode of action, as distinguished from a mechanism of action, reflects a mathematical approximation that does not require an understanding of the molecular level. It basically requires the mode of action to be biologically plausible and that it fit mathematically.

A number of important concepts for the development of dose-response curves can be shown through a comparison of the assumptions implicit in a horizontal, vertical, and diagonal dose-response function. In the horizontal function example, susceptibility is 0 or 100%, any dose is lethal if susceptible and a host is either 0% or 100% susceptible. In the vertical function example, all have the same resistance and the lethal dose is the same

for all. However, not all doses are lethal. For the more typically presented diagonal dose-response line, the risk is proportional to the dose (e.g., carcinogenic radiation).

The probit curve is an example where a population's hazard resistance is normally distributed and the dose-response relationship follows a cumulative normal distribution (Figure 7). Here the individuals who will exhibit a response at a given dose follow the probit model (cumulative normal distribution). However, it should be noted that multiple parameters determine resistance.

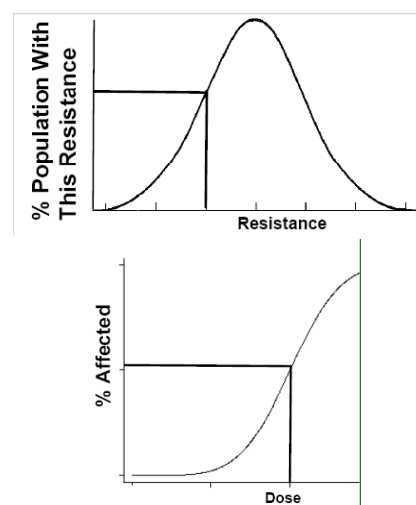


Figure 7. When a population's resistance is distributed normally, the resulting dose-response curve is a cumulative normal distribution (i.e., probit curve).

Variability is another important concept in dose-response modeling. Variability in dose-response is the result of individual differences in resistance. With perfect information, all dose-response queries can be answered with certainty and models are essentially deterministic (e.g., diagonal line model example). Uncertainty in dose-response is a result of the element of chance. Chance can be described as stochastic, or probabilistic. In these scenarios, even perfect information will never allow for certainty in the answer because of the influence of chance (e.g., radioactive decay). The communication of uncertainty when describing results should distinguish between uncertainty and variability, and provide an analogy that will help explain the model results.

Decision science can be used to inform development of models. The starting point should be an identification of the real-world decision(s) to be made using the model and the drivers for the decision. To make decisions in a well informed manner, the theoretical and empirical considerations as well as qualitative and quantitative information should be considered. To determine appropriate models for use, biological plausibility and validation with empirical data should be used.

Microbial Dose-Response Methods Comparisons – Benchmark Dose Approach

**Jeff Gift, Ph.D.
EPA**

The benchmark dose (BMD) modeling approach involves the application of empirical modeling (mathematical curve fitting) methods to available data (e.g., dose-response data for a given toxicological endpoint). One advantage of empirical modeling is that it is intuitive and it relies on all of the dose-response data to derive a risk assessment point of departure (POD). A disadvantage is that such empirical approaches can provide very different curve fits in the low-dose region of the dose-response depending on the selected model. There can be high uncertainty in these estimates with respect to both the accuracy and biologically plausibility of the results. As a result, in the application of BMD methods, risk assessors need clear guidance that takes these uncertainties into account. There are published recommendations on determination of a model fit for available data (i.e., Haas, Rose, and Gerba, 1999). There is considerable overlap between the above referenced book and the guidance contained in EPA's BMD Technical Guidance (U.S. EPA 2000) and Benchmark Dose Software (BMDS) (U.S. EPA, 2009).

Considerations in the process include evaluation of the best parameter estimates for a given dose-response model, determination of an adequate model fit, approaches to determine among a set of plausible models which model best fit the data, evaluation of uncertainty in parameter estimates and the benchmark response (BMR) level from which to derive a benchmark dose POD. The U.S. EPA's BMDS (U.S. EPA, 2009)¹ and BMD technical guidance documentation (U.S. EPA 2000) provide a set of tools and procedures for making these determinations.

BMDS is an open source platform that facilitates the application of BMD methods by fitting the mathematical curves to dose-response equations. The BMDS can run a suite of models and the results can be compared in tabular or graphic form. The evaluation includes an AIC value (Akaike's information criterion)², goodness of fit measure (p-value), calculated benchmark dose and benchmark dose level. Chi-square residuals are also available for dose groups, including those of lower doses (the area in the dose-response that is generally of greatest concern) in the data set. EPA's BMDS website (www.epa.gov/bmds) contains training materials and

flow charts which walk users through the process of doing a BMD analysis including determining the most appropriate BMR and the best fitting model. The BMD approach as it is applied by EPA has an additional advantage; it accounts, in part, for the quality of the study (e.g., study size) by estimating a BMDL, the 95% lower bound confidence limit on the BMD. The BMDL is closer to the BMD (higher) for larger studies and further away from the BMD (lower) for small studies. Thus, the BMDL accounts, in part, for a study's power, dose spacing, and the steepness of the dose-response curve.

The BMDS can accommodate current data gaps. For example, low dose extrapolation for cancer dose-response assessment and microbial risk assessment carry some of the same challenges. In this case, policy determinations have been made to assume linearity or nonlinearity, and to use this as extrapolation mechanism.

EPA has also developed a set of categorical regression models, CatReg 2009 R version (CatReg) that may provide assistance in addressing some of the data extrapolation gaps in question. The software has been built to run on an R platform and the software is open source (<http://www.epa.gov/ncea/catreg>). Categorical regression allows for the use of categorical responses to be modeled, with time and intercept parameters, which could allow the data to be pooled and the probability of getting x- responses at a specified severity to be calculated. CatReg can also be used to evaluate response over different time durations. CatReg also allows for the stratification of dose-response data (e.g., by species, sex or strain) so that the contribution of each stratification to the overall model fit can be estimated.

¹ At this time, BMDS offers over 30 different models that are appropriate for the analysis of dichotomous, continuous, nested dichotomous and time-dependent toxicological data.

² Akaike's Information Criterion (AIC) (Akaike, 1973) is used for model selection and is defined as $-2L + 2P$ where L is the log-likelihood at the maximum likelihood estimates for the parameters and P is the number of model degrees of freedom.

Session 5: Dose-Response Applications for Vaccines and Therapeutics

Presenters were asked to address the following stimulus questions:

1. How are biomarkers utilized in dose-response modeling of infection and/or disease?
2. Can dose-response thresholds be estimated for vaccines and/or therapeutics?

Dose-Response Applications for Vaccines & Therapeutics

Conrad P. Quinn, Ph.D.
CDC

Biomarkers of infection and disease are valuable tools for formulating an earlier diagnosis, informing patient management, and monitoring therapeutic intervention and disease progression. Dr. Quinn presented his current research on measuring exposure to *B. anthracis* and understanding the potential clinically detectable biomarkers of exposure, infection, and disease.

Exposure to environmental *B. anthracis* may be innocuous because of the protection afforded by host intact immune barriers or may elicit an innate host response; exposure does not necessarily result in infection and subsequent anthrax disease. Disease progression from infection is dependent upon spore uptake and germination; this is a secondary key step to the breach in the host intact immune barriers. Potential host biomarkers for environmental *B. anthracis* exposure and infection include host responses associated with, lethal factor, protective antigen (PA), and capsular γ -linked poly-D-glutamic acid. Anti-anthrax trisaccharide is antigenic, exposed on the surface of spores, and contains a *B. anthracis*-specific epitope. Lethal factor toxemia is specific to *B. anthracis*, is quantifiable in serum/plasma and, in a rhesus macaque model of inhalation anthrax, becomes detectable approximately 12-18 hours after an initial spore aerosol exposure. Seroconversion to anti-PA immunoglobulin G (IgG) is also a host biomarker for anthrax. During the anthrax letter attacks of 2001, anti-PA serology was a contributing test in the confirmatory diagnosis of 12/22 cases, critical to the diagnosis in 6/22 cases and the single confirmatory test in 3/11 cutaneous anthrax cases. General trends that are consistent with survival observed in experimental animals as well as one human case include decreases in lethal factor with a concomitant increase in anti-PA IgG.

Dr. Quinn proposed several issues for further discussion. What should be the dose-response threshold for biomarkers of exposure, infection, and seroconversion responses? With current levels of knowledge, the

dose-response modeling may not be sufficient for curve determination; it may be appropriate to consider the development of a toolbox to begin to address this data gap. Regarding the dose-response thresholds for therapeutics – what is the timeframe post-exposure within which the drug is effective? Is there a point of no return? Could certain treatments actually exacerbate disease?

Dose-response predictions can be estimated for vaccines and therapeutics. For vaccines, field efficacy and immunogenicity studies are part of vaccine development. Combined measures of experimental models can assist in evaluating vaccines to ensure effectiveness. There are a number of measures for therapeutics which are routinely conducted as part of product testing including pharmacokinetics, therapeutic indexes, and therapeutic window determinations.

How are Biomarkers Utilized in Dose Response Modeling of Infection and/or Disease?

Louise Pitt, M.D.
US Army Medical Research Institute of Infectious Diseases (USAMRIID)

One of USAMRIID's current overarching research goals is the development of well characterized animal models for aerosol exposures of biological threat agents. By developing these animal models of disease, appropriate biomarkers can be identified that will inform the proper timing of effective therapeutics and/or vaccines. Biomarkers can be critically important in aiding early diagnosis and useful in the identification of therapeutic targets for treatment, and they can be detected at various molecular or clinical investigative levels. Molecular measurements can include microarray analysis, proteomics, and metabolomics, while clinical measures can include bacteremia, viremia, hematology, chemistries, cytokine levels, temperature, immune response, and toxemia. For the valuable use of a particular biomarker, it is preferred that there is a rapid assay available to detect the biomarker and that there is good correlation between the biomarker and disease. The biomarker should be evident in the relevant animal model; the disease process in the animal has to mimic human disease and the pathogenesis should be well understood. The biomarker must not be pathogen strain-specific, must be identifiable, and must have similar expression regardless of strain.

To allow for estimation of dose-response thresholds for vaccines or therapeutics, "humanized" vaccine or therapeutics doses and schedules must be developed and used in animal challenge studies. This allows for the potential extrapolation of animal efficacy data to human efficacy. Thresholds can be determined by increasing the

challenge dose until breakthrough illness is observed; however, increasing the challenge dose is technically challenging for many biological threat agents. An additional means to test for vaccine thresholds is to reduce the vaccine dose and/or schedule while still maintaining the same challenge level. It is anticipated that the test animal would mount an incomplete response; this information is then used to develop correlates of immunity. A third method is to assess host species susceptibility so that agent virulence can be evaluated across species and to assess the durability of immunity. Knowledge of the species-specific immune responses can assist in making determinations regarding the durability of immunity. For example, rabbits and nonhuman primates present a similar disease course, but they exhibit different rates of disease progression and differences in the duration of immunity. IgG predominates in the immune response of the rabbit, and therefore, immunity does not persist as long in rabbits as nonhuman primates.

Dose-Response: Economics and Public Policy (or, the value of risk)

Martin Meltzer, Ph.D.
CDC

From the perspective of the policy maker, dose-response analysis is all about the risk for the endpoint of concern. The role of modeling for policy development is to inform about potential trade-offs; for example, what are the side-effects of spending resources? Is it possible to maintain zero risk or the “perfect” vaccine or drug? If so, what are the costs and side effects? Policy decisions such as these are faced all the time in the public health field. For example, a public health policy decision was made as to whether to recommend vaccines to those who were exposed to the anthrax letters. Available data on spore survival showed that spores could survive *in vivo* perhaps up to 60 days, though in potentially small numbers. The policy decision was to *not* recommend the vaccine. It was assumed that the risk of disease was dependent upon the duration of antibiotics, and a 60-day course of antibiotics was recommended instead. In retrospect however, it was found that overall adherence to antibiotics was poor; only 44% of those prescribed antibiotics took them for the fully recommended duration. This poor adherence was mainly due to the gastrointestinal side effects of the prescribed antibiotics. One way to look at this is that the risk-versus-tradeoffs valuation changes over time, with newly available information or personal experience. Even though in the beginning those exposed initially wanted the antibiotics, this original desire was modified by the experience of the gastrointestinal side effects.

Pre-exposure smallpox vaccination is another area where risk-benefit tradeoffs pose difficult challenges for

public health policy makers. Results from a survey of the general public show that approximately 61% of the public would desire and accept smallpox vaccination if it were offered. The risk of smallpox is a function of the number initially infected, the probability of release, probability of contact, probability of transmission, and vaccine effectiveness. The serious vaccine adverse effects are a function of the probability of side effects. However, it is only when the risk of smallpox is greater than zero that pre-exposure vaccinations should be considered (Figure 9). It was found that with a 1:10 risk chance of 1,000 smallpox cases in a potentially exposed population of 280,000,000 people, an individual would have a greater risk of vaccine related adverse effects than risk of contracting smallpox.

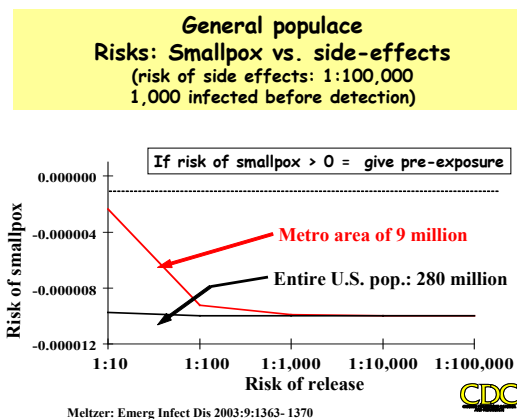


Figure 8. Risk of smallpox release relative to risk of smallpox infection for two different exposed population sizes.

Dr. Meltzer concluded that the utility of using models is not always to produce “a number”; the concepts contained in the model are as important as the data/numbers. It is more important to understand what is being built and why. Models can also be useful because they highlight data deficiencies, “how thin is the ice” on which decisions are being made. Model development should balance between simplification and being simplistic. Utility is improved when one model can be used for a limited set of questions. Utility is also maximized when changes in results can be demonstrated with changes in assumptions. Confidence intervals are essential in describing outcomes and associated probabilities. Model utility is improved when probabilities for outputs can be described.

Workshop participant and presenter discussions were held following each session throughout the two and a half days. The following section summarizes the main points according to overarching discussion topics; it is not a complete record of all detailed discussions, nor does it embellish, interpret, or enlarge upon matters that were incomplete or unclear.

Group Discussion Summaries

Microbial Dose-Response Modeling

Several general dose-response modeling concepts were presented by workshop attendees. The best dose-response models all have flaws – as they are, at best, a simplification. The key to the dose-response assessment is to determine what can be done to make the models useful and to recognize that the models do not need to be perfect. Multiple models will most likely be necessary to meet the challenges and decisions. The critical focus for model selection is who is making what decision and why; the model should be built and utilized to inform the decision.

There are many different variations of dose-response models, and assessors will have to select the appropriate model for the required decision. The assumptions for the model must be realistic and clearly understood, and model results must be consistent even when the assumptions are relaxed. As the model increases in complexity, a more detailed estimate can be gained; however, there will also be a significant increase in the model uncertainty. The model needs to have the “right” complexity; very often there are too many parameters in the models. There must be a deliberate effort to choose the appropriate number of parameters in the dose-response model while avoiding confronting the issue of forcing the data to fit. There was a continued discussion regarding whether to let the data determine the dose-response model selection or to modify the data to fit the model. Overall, it was agreed that the models should be, if possible, biologically plausible for the specific pathogen exposure and the data should have a statistically significant fit in the model. This point of discussion as well as others throughout the workshop was highlighted along with the notion that there are opportunities to develop microbial dose-response modeling approaches by learning and utilizing what has been done in chemical dose-response assessments.

Mechanistic-Enough

Several discussions focused on the differences between mechanistic and empirical dose-response models. For termed mechanistic models, it was questioned whether these models are “mechanistic enough.” The beta-Poisson dose-response model is termed a mechanistic model as it is thought to be based on biological plausibility; however, there was general consensus that to better inform risk assessments, models need to include mechanisms behind dynamic processes and not just distributional processes as is described with the

beta-Poisson dose-response model. Risk assessment requires evaluations that indicate the adequacy of a model for the particular assessment being performed and this, in turn, requires clarification of assumptions made regarding processes involved in generating the data and determining outcomes. A model where these assumptions are obscure is insufficiently mechanistic. Furthermore, too much mechanistic detail could burden assessments with excessive degrees of parameter uncertainty. Consequently, microbial dose-response models should be just “mechanistic enough.”

Thresholds

The issue of thresholds in microbial dose-response modeling was addressed by several participants with varying opinions. For example, some were of the opinion that there can be no threshold when only one organism has a probability of infection. It was questioned that if a threshold were present in the data, would it be discernable using the currently available dose-response models? These single hit dose-response models may not be acceptable for all pathogens and all endpoints. Most agreed that the existence of a threshold would have to be investigated on a pathogen-specific case-by-case basis. For example, the human immunodeficiency virus requires exposure to high viral numbers that reach the mucosal surface; infection, however, is initiated by one cell. In contrast, there are other microorganisms that must act in concert to initiate infection (e.g. quorum sensing).

Several individuals felt that the key processes or steps of the infection cycle (invasion, infection, illness) each have the potential for a dose threshold. Within each of these steps, there are potential barriers that can influence the threshold dose required to reach the next step. The problem is that it is difficult to discriminate between these key processes and to identify and isolate the appropriate endpoints of invasion, infection, and/or illness caused by a single pathogen. To address this need for potential endpoints, generation and verification of more mechanistic data are required.

Biomarkers

Biomarkers can be used in dose-response and potential threshold modeling. However, it was noted that they will most likely be disease-specific and therefore their intended use will also be specific. The biomarker of interest will be dependent on the endpoint being modeled and what response the biomarker is designed

to predict. For example, the biomarker for infection may be different than the biomarker for early diagnosis of disease aimed at successful treatment intervention.

Considerations of Exposure Assumptions for Microbial Dose-Response Modeling

Throughout the workshop, participants noted that it is difficult to completely separate the exposure assumptions from dose-response modeling. In addition, a thorough understanding of the role of the environment in exposure is necessary to better define the contexts in which a microbe exhibits pathogenicity. For example, naturally occurring *Bacillus anthracis* in the pasture is not typically considered to be highly hazardous compared to intentionally-released *B. anthracis* in a building. Furthermore, for some microbes, the definition of pathogenicity is highly dependent upon the host being invaded. There are a number of microbes that may be pathogens to individuals with compromised immune systems, but may not be pathogenic to those with competent immune systems.

Assessors also need to be attentive to the actual reported measured doses. Microbial dose-response data are challenged by a rather limited ability to measure extremely low doses combined with an inability to tell whether the challenge presented was viable or not. For example, one referenced study delivered dose was 10 oocysts – which turned out to actually be 10 +/- 4 oocysts. Another issue with the historical data sets, specifically for inhalational exposures, is that the particle size was typically unknown. Particle size greatly impacts the total internal doses and thus will impact the dose-response estimates.

Variability in concentration is another important consideration in both sampling and dose-response modeling. Outbreaks may occur from outliers in doses for a given medium. A concentration that is acceptable if homogeneous, may pose a hazard when present in hotspots of higher concentration and areas of lower concentration. The average concentration is the same, but the individual exposure doses can be considerably higher.

Furthermore, historical assessments have typically assumed microbes are Poisson-distributed in the environment. However, it was noted that this assumption has not always held up in environmental and laboratory samples. Microbial environmental samples typically present as a skewed distribution, and it is only when the sample is a well-mixed sample from a laboratory that a Poisson distribution may be able to be appropriately assumed. However, it was argued that there is published *Cryptosporidium* data obtained from sampling of a

pristine water body that conclusively demonstrated that environmental samples are Poisson distributed.

Pooling Microbial Dose-Response Data

The workshop attendees also considered the appropriateness of pooling microbial dose-response data. The first question that was asked during the discussion was “why” pool the data; some participants felt that data pooling can be very “tricky” and may not be appropriate as it will raise red flags for most decision makers. The example of pooling various experimental animal species dose-response data was considered. One of the biggest obstacles stated with this example is how to reflect and account for the potential differences between the species in the various parameters. It may be appropriate to pool data between species, but how is this type of analysis communicated clearly to the decision makers with accompanying assumptions. Ultimately, pooling data could allow more information to be gathered together to make stronger inferences if differences in data sets were accurately reflected and adjustments were made.

Microbial Dose-Response Modeling Extrapolations

Throughout the workshop, there were many discussions as to the utility of the dose-response models and the need to extrapolate data. Almost always, the decisions made from dose-response modeling efforts involve extrapolated data. Three types of extrapolations were discussed: animal-to-human, high-to-low doses, and healthy-to-sensitive subpopulations.

Animal-to-Human Extrapolations

There was a clear understanding and agreement between the attendees that one of the greatest challenges with microbial dose-response modeling is the very limited availability of human dose-response data. As a result, the majority of dose-response estimates rise from experimental animal studies. In the best case scenario, the animal models used for human exposures should meet the following assumptions:

- 1) The disease is caused by the same mechanism from the same agent with a comparable progression and time course.
- 2) There are similar immunological and physiological responses, signs, and symptoms in the animal model and human.
- 3) The animal model provides the ability to quantify information on levels of infection, morbidity, and mortality.

To account for the uncertainty in the animal-to-human extrapolations, the question of the appropriateness of scaling and/or uncertainty factors was raised.

Uncertainty factors are widely used in chemical dose-response modeling and could be potentially valuable for extrapolating microbial data to estimate human exposures. It was noted that magnitude and degree of application of these uncertainty and/or scaling factors should depend on the decision to be made, the specific situation, and the endpoint of concern.

Physiological-Based Modeling

To more accurately decrease the uncertainty from the animal-to-human extrapolations of dose-response data, assessors can utilize species-specific physiological-based models. Physiological-based models are a relatively new approach for microbial risk assessment; however, these detailed models have been widely used for many chemical dose-response assessments. It was noted that physiological-based models can be very data hungry and thus very expensive to advance. These complex models need data for many different parameters as there are many physiological interactions between the host and pathogen that could be modeled.

For the important pathogens of concern, there is significant interest and potential utility in developing pathogen-specific physiological-based models. However, for the majority of microbial agents, there may be value in identifying commonalities among the complex systems that are part of these models. For example, intracellular microbial pathogens could be grouped and considered by one type of model with parameter modifications for agent- or host-specific characteristics. *Bacillus anthracis* and *Francisella tularensis* have similar characteristics that may allow for generally similar models to be used with some re-parameterization to fit them.

Several participants questioned how these physiological-based models can be verified. It was noted that the model predictions can only really be fully tested in animals; however, some of the various parameters of the models could be verified with human *in vitro* studies. Another approach considers if these advanced models can be predictive for - and then tested in - other experimental animal species; if the model can accurately extrapolate responses from animal-to-animal, then the model should be able to then predict more accurately and extrapolate from animal-to-human.

High-to-Low-Dose Extrapolations

Another challenge with modeling microbial data from dose-response studies is that most historical studies administered extremely high doses to achieve effects, and therefore, the data require extrapolation from high-to-low doses to predict potential human responses at low doses. There can be large orders of magnitude differences in dose-response curve estimates in the

resulting low dose extrapolations depending on the type and amount of data being modeled. It was noted that the interest is not necessarily with the low-doses; the focus is really about low responses and probabilities. However, studies are not typically conducted in the very low probability area of the dose-response curves, therefore, most studies, at best, focus on the “middle” portion of the data such as the lethal dose that caused death in 50% of the test population (LD_{50}).

Participants also noted that while many organizations have indicated an interest in primarily the low dose region of the dose-response curve, the Department of Defense also has an interest in mid to high level doses as well as the low dose responses. Risk is characterized on a sliding scale from negligible to catastrophic in recognition of acceptable losses and of the potential for mission importance to overcome adversity to risk. On the other hand, EPA has the issue of determining low dose-response relationships that will be applied to chronic low dose exposures (i.e., multiple doses) for remediation goals applicable to re-occupancy scenarios.

Healthy-to-Sensitive Subpopulations Extrapolations

There was a great deal of discussion regarding sensitive subpopulations among the workshop participants. It was questioned whether the focus of dose-response modeling should estimate sensitive subpopulations or is it adequate to assess the risk for the majority of the population. Most models assume a homogenous human population and generally do not account for disease impact on sensitive subpopulations. For example, *Listeria* outbreaks in Europe have demonstrated that current dose-response models are underestimating the hazard to those 60 years and older. There was a general consensus that it may be appropriate to evaluate different dose-response models based on sensitive subpopulations as well as the majority “healthy” population.

The discussion next focused on how best to extrapolate the dose-response data from “healthy” individuals and/or experimental animal species and then apply the data to the larger population to account for the presence of potentially sensitive subpopulations. In chemical risk assessment, the approach has been to model the “healthy” populations first and then to use uncertainty factors to account for the large population including the sensitive subpopulations. However, it was recognized that one study will most likely not be adequate; it will be knowledge gained from multiple dose-response studies and outbreak data as was mentioned with the example of the *Listeria* outbreaks in Europe. Outbreak data can be particularly helpful for comparisons of the response in the healthy populations with the response in potentially sensitive subpopulation (e.g., children, elderly).

Communicating Microbial Dose-Response Modeling Results

Discussions also centered on effective communication and interpretation of dose-response modeling results to improve microbial risk assessment and mitigation practices. The participants acknowledged the importance of planning for communicating modeling results and the uncertainties associated with these results. It was noted that the relative effort that was typically spent in building models did not include enough planning for communication of model characteristics and results. Attendees suggested that it was best to combine the risk communication process with the risk assessment/risk management process early in the planning and to do so often during the entire process.

Communication of modeling results and risk should be done in a manner that supports policymakers in their decision making approach. It is also important for the decision makers to try and bridge the gap by understanding the underlying science. Dose-response modelers need to communicate the assumptions, strengths and weaknesses of their models up front so that risk managers can interpret and apply the models correctly. Additionally, it is important to express uncertainties in the numbers generated and include confidence limits to explain confidence and probability of illness. It should also be noted that for more meaningful results, it is important to look at the total distribution. The confidence in the data at any given point depends on the overall meaning and the confidence associated with that particular point in the distribution.

Risk communication approaches need to be tailored to the respective audiences and accompanied by well designed translation functions for specific audiences. For example, when communicating to the large public, there is not a likely difference in public perception of a 45% versus 55% chance of getting ill. In fact, there is no single “threshold” of public concern that should be considered to be present. The percent of concern changes every time and is highly dependent upon the specific circumstances and potential consequences. The percentage is not the key to public concern; it is the public’s understanding and acceptance of risk greater than 0%, and the required learning curve for the understanding of novel public health threats by the public.

Microbial Risk Assessment Standard Terminology

Several participants noted that developing standardized microbial risk assessment terminology would be very valuable and would facilitate successful collaborations across disciplines. Communicating methods and results between the disciplines has been difficult at times

as the various disciplines sometimes apply different terms to define the same approach or the same term to define different approaches. For example, the term “mechanistic” was used to describe both empirical dose-response models and physiological-based models even though these methods are two very different approaches to modeling. Others disagreed with developing standardized terminology and felt that it was sufficient to emphasize clear communication and associated definitions while presenting and discussing work efforts.

A new term for microbial dose-response modeling, the lowest observable tolerable environment level (LOTEL), which is conceptually similar to a NOAEL or LOAEL type measure, was also discussed. The basis for considering this new term is to describe the lowest “tolerable” dose that can be identified for the endpoint and receptor of concern. From a toxicological perspective, NOAELs and LOAELs have been successfully used in chemical risk assessment. However, given the current microbial dose-response models in use, it may not be possible to identify how “tolerable” might be defined. Furthermore, it is critical not to intertwine the actual science data with the science policy and perception. Participants agreed that care should be taken prior to usage and acceptance of a new term.

Application of Microbial Dose-Response Modeling

One goal of the workshop was to address how to use dose-response data to support the derivation of risk-based remediation goals following the release of a biological agent.

There were discussions on developing risk-based goals that provide direction on the selection of steps to minimize risk for post-event and re-occupancy decisions, on the use of antibiotics post-exposure, and on providing an alternative to a zero or no-growth cleanup goal. The discussion about remediation and re-occupancy goals was limited to the cleanup of aerosolized *B. anthracis* spores.

The following questions were used to guide the discussion:

- What endpoint is sufficient for remediation and re-occupancy?
- Can a cleanup goal of zero viability actually be achieved with current decontamination technologies?
- Should cleanup be to the background level of the agent in the environment?
- Is there a dose of *B. anthracis* spores that an immuno-competent individual can tolerate without advancing to disease?

- How does the detection limit of the analytical methods capabilities affect achievement of the cleanup goal?
- Should the extent of cleanup maximally achievable with employment of engineering controls with the best available technology be considered in the derivation of a cleanup goal or should only the health risk of exposure be the driver?

Definitive answers to most of these questions require more research, but some answers in the interim will be based on the scenario at hand. Attendees gave insights on how response and remedial actions have been employed with other agents and other environmental media. In general, the control of pathogens in drinking water has been through the use of treatment or technologically based standards. Health-care facilities utilize infection control practices by focusing on blocking transmission with engineering controls to minimize risk of exposure to pathogens. Similarly, the food industry has implemented the Hazard Analysis and Critical Control Point (HACCP) system as an effective and rational means of assuring food safety from harvest to consumption; preventing problems from occurring is the paramount goal underlying any HACCP system.

Conclusions and Future Steps

This report was prepared as a summary of the presentations and discussions held at the EPA/CDC *State-of-the-Science for the Determination and Application of Dose-Response Relationships in Microbial Risk Assessment* Workshop, April 21-23, 2009. Participants of the conference shared knowledge, explored differing opinions, and expanded overall understanding in MRA dose-response relationships. Because of the diversity of attendees' disciplines, the different inputs and decision making required to support each organization's mission, and the limited timeframe, the primary goal of the conference was to share knowledge, explore differing opinions, and expand overall understanding in MRA dose-response relationships. The report captures the main points and highlights of the meeting, but does not embellish, interpret, or enlarge upon matters that were incomplete or unclear.

As a follow on to these discussions, a third MRA conference/workshop is being planned for 2011. This conference will be held to focus on the exposure assessments of MRA.

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Appendix A: Workshop Agenda

Day 1: Tuesday, April 21, 2009

- 8:00 – 8:30 a.m. Registration
- 8:30 – 9:00 a.m. Welcome - *Cynthia Sonich-Mullin, U.S. Environmental Protection Agency (EPA)*
- 9:00 – 9:30 a.m. Keynote Address: “MRA Dose-Response Challenges”
Cynthia Chappell, University of Texas School of Public Health
- 9:30 – 9:45 a.m. Participant Feedback and Discussion
- 9:45 – 10:05 a.m. Break
- 10:05 – 11:30 a.m. Federal Mission Needs for Dose-Response
Tonya Nichols, EPA
Michael Bell, Centers for Disease Control and Prevention (CDC)
David Oryang, Food and Drug Administration
Janell Kause, U.S. Department of Agriculture
- 11:30 – 12:00 p.m. Participant Feedback and Discussion
- 12:00 – 1:00 p.m. Lunch
- 1:00 – 1:30 p.m. Dose-Response Extrapolations
1. How is uncertainty and variability addressed in extrapolating dose-response data (e.g., extrapolating across host species, exposure levels, routes of exposure, durations of exposures, pathogen strains or species, endpoints, and/or sensitive populations)?
 2. Is it appropriate to group studies, animal models or host species, and/or pathogen strains or species in dose-response modeling of multiple data sets?
- Margaret Coleman, Syracuse Research Corporation*
Charles Haas, Drexel University
Thomas Whalen, Georgia State University
Mary Alice Smith, University of Georgia
- 1:30 – 2:30 p.m. Participant Feedback and Discussion
- 2:30 – 3:00 p.m. Break
- 3:00 – 3:30 p.m. Physiological-Based Modeling
1. What overall assumptions are necessary for valuable physiological models to predict human consequences?
 2. What is the minimum data set required (i.e., what level of detail needs to be modeled for acceptable human predictions (e.g., whole species models, organ-specific models, and/or cellular or toxin activity models)?
- Sarah Taft, EPA*
Jeff Gearhart, The Henry M. Jackson Foundation for the Advancement of Military Medicine
Michael Lumpkin, Syracuse Research Corporation
- 3:30 – 4:30 p.m. Participant Feedback and Discussion
- 4:30 – 4:45 p.m. Preview of Day 2

Day 2: Wednesday, April 22, 2009

8:30 – 8:45 a.m. Day 2 Opening Remarks

8:45 – 9:45 a.m. Dose-Response Method Comparisons:
Classical, Bayesian, Epidemiology, and Benchmark Dose Modeling

1. Is the dose-response statistical method utilized empirical or mechanistic?
2. Is the method applicable for low-dose extrapolations?
3. Can the method accommodate data pooling and/or the use of correction factors?
4. How is the calculated dose-response relationship verified and validated?
5. How is model uncertainty adjusted for and communicated to risk managers?

Classical – Tim Bartrand, Clancy Environmental Consultants

Bayesian – Jade Mitchel, Blackwood, Drexel University

Epidemiology Modeling – Laurie Waisel, Concurrent Technologies Corporation

Benchmark Dose Modeling – Jeff Gift, EPA

9:45 – 10:05 a.m. Break

10:05 – 11:45 a.m. Participant Feedback and Discussion

11:45 – 12:00 p.m. Closing Comments from Stimulus Presenters

12:00 – 1:00 p.m. Lunch

1:00 – 1:30 p.m. Stimulation Activity: Going Beyond the Dose-Response Curves!

1:30 – 3:45 p.m. Breakout Activity (4 teams)

3:45 – 4:45 p.m. Teams Report Back

4:45 – 5:00 p.m. Discussion on Playback Reports

Day 3: Thursday, April 23, 2009

8:30 – 8:45 a.m. Day 3 Opening Remarks

8:45 – 9:15 a.m. Dose-Response Applications for Vaccines and Therapeutics

1. How are biomarkers utilized in dose-response modeling of infection and/or disease?
2. Can dose-response thresholds be estimated for vaccines and/or therapeutics?

Conrad Quinn, CDC

Louise Pitt, U.S. Army Medical Research Institute of Infectious Diseases

Martin Meltzer, CDC

9:15 – 10:30 a.m. Participant Feedback and Discussion

10:30 – 11:00 a.m. Next Steps

11:00 a.m. Adjourn

Appendix B: List of Participants

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Appendix C: Slide Presentations

Coleman:

To supplement the Coleman summary in Session 2, the following is a more detailed description of the specific pathogen examples presented:

Anthrax: Aerosol challenge of rodents with *Bacillus anthracis* may be misleading for primates with different patterns of deposition due to anatomical and physiological differences in respiratory systems. *In vivo* images were generated in mice challenged by different routes with high doses of a bioluminescent nontoxigenic capsulated strain of *B. anthracis* (aerosol 108; intranasal 105; intratracheal 105; intravenous: 106 to 107; images from Glomski et al, 2007). Spores depositing in the turbinates of rodents infect nasal cavity and throat tissues before the lung, whereas spore deposition in human respiratory tract system is deep in the lung due to differences in anatomy and physiology. Rodents also swallow inhaled particles, and resultant gastrointestinal pathology in mice may be poor predictor for human effects by inhalation route. Therefore, rodents may not be reliable predictive models for inhalation anthrax and other human respiratory diseases. If rodent models are to be useful for predicting human effects, deposition and clearance models are needed for scaling doses and translating system level knowledge.

Similarly, the relevance of mice to humans for oral and dermal challenges with *B. anthracis* merits further investigation and analysis. For gastrointestinal anthrax, knowledge is so sparse that this demonstration of tropism to Peyers' patches in mice is relevant (intragastric catheters or feeding needles at 108), as are conflicting results from other animals resistant to high dose challenges (guinea pig, rabbits, rhesus at 108 spores; dog, guinea pig, sheep at ~105). Future mechanistic models may explain these inconsistencies and provide more robust decision support for preparedness planning.

For dermal anthrax, systemic involvement in rodents from sub cutaneous challenge (injection into dermis of ear (500 or 10,000 spores)) is atypical of human cases of cutaneous anthrax, largely localized infections via damaged skin.

Historically, human cases of gastrointestinal and cutaneous anthrax are associated with animal outbreaks in hyper-endemic regions of the world. Epidemiologic investigations report human cases occur in proportion to animal cases. Approximately one human gastrointestinal case per 30-60 animal cases and approximately one

human cutaneous case per ten animal cases were associated with consuming, preparing, or butchering meat from contaminated carcasses during epidemics of anthrax (Turnbull, 2002). The paucity of human cases in the US, despite outbreaks in livestock and wildlife, may be due to more effective interventions (e.g., vaccination, protective equipment) and inspection procedures to keep diseased animals out of the US food supply.

Sound dose-response assessments must incorporate knowledge of the mode/mechanism of anthrax in animals and humans for robust extrapolations.

Salmonellosis: Outbreaks in peanut butter provides a great example for discussion of extrapolation because available animal and human data alone do not directly address susceptibility of children, 3-13-fold more susceptible than older age groups as reported in a 2003 FoodNet study.

Consider a family of dose-response curves from murine studies conducted in the 1950s and 1960s by Bohnhoff and colleagues. Fifty percent of normal healthy animals are infected at approximately a million *Salmonella enteritidis* cells. The dose-response curve is left-shifted five orders of magnitude to an ID50 less than ten cells when animals are rendered more susceptible by treatment with antibiotics that disrupt the normal protective effect of the indigenous microbiota of the gastrointestinal tract. Susceptibility in treated mice returns to normal as the interval between antibiotic treatment and pathogen challenge increases to five days and the protective effect of the indigenous microbiota is restored.

Direct use of the murine curves for humans is not recommended due to the systemic disease pattern in mice, atypical of human disease. However, the existence of rich human dose-response datasets for salmonellosis and a species-common mechanism, antibiotic disruption of the protective effect of the indigenous gut microbiota, permits scaling of mouse and human dose-response relationships to reflect variability in susceptibility within and between hosts.

Much of the work with the human salmonellosis datasets was published with my collaborator Harry Marks at USDA. Nearly 400 human volunteers were challenged with 13 strains of *Salmonella* (McCullough & Eisele, 1951). Accounting for strain differences by ANOVA, nine strains can be pooled, but not with the four *Salmonella pullorum* strains with a different

mode of action consistent with a statistically significant threshold dose-response relationship (Coleman and Marks, 2000). ANOVA models provided significant fit for multiple empirical models, with different low-dose behaviors. Both models fit the data well in the observed region, but differed by >75 order of magnitude when extrapolated to the dose of a single *Salmonella* cell. Model uncertainty and strain variability are obviously significant for salmonellosis. The Weibull model for human salmonellosis was scaled to the murine family of curves to generate a family of curves that represent human populations of increasing susceptibility based on the protective effects of the indigenous microbiota common to mice and humans. Strain variability can also be described for the most susceptible host, with inflection points ranging from 1 or 1000 salmonella cells that could cause illness in susceptible human hosts, based on the available murine and human data.

To select models from these families of empirical dose-response curves that are representative of infants, children and adults, mechanistic knowledge and models, as well as target *in vitro* or *in vivo* research, are necessary to further illuminate the key events in host-pathogen interactions for appropriate scaling.

****Note to Reviewers: The following section contains presentation slides that were approved by the presenters for inclusion in this document.*

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Microbial Risk Assessment for the Development of Cleanup Goals

Tonya Nichols, Ph.D., Acting Associate Director
Threat and Consequence Assessment Division
National Homeland Security Research Center
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Cincinnati, Ohio, U.S.A



SCIENCE

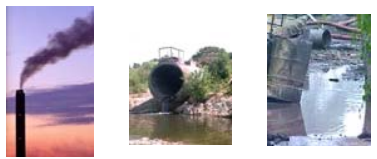
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April, 2009

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EPA's Mission:

To protect human health and to safeguard
the natural environment – air, water, and
land – upon which all life depends



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The Problems We Face

- Protecting against environmental contamination
- Determining that a release has occurred
- Containing contamination
- Mitigating impacts
- Assessing and communicating risk
- Decontaminating impacted areas
- Disposing of contaminated materials

...EPA conducts risk assessment
to inform risk management decisions

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Risk Assessment → Risk Management U.S. EPA Cleanup Goals

Superfund program

- Preliminary Remediation Goals (Soil and Water)

Office of Water

- Health Advisories
- Maximum Contaminant Levels

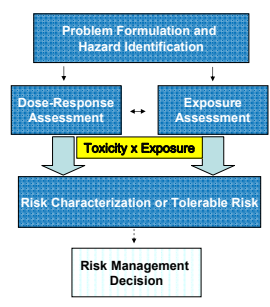
Office of Air Quality Planning and Standards

- Reference concentrations
- Inhalation unit risk

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Risk Assessment



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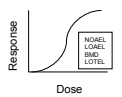
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Risk Assessment → Risk Management U.S. EPA Cleanup Goals

Exposure x Toxicity = Chemical Risk

Exposure x Pathogenicity = Biological Risk

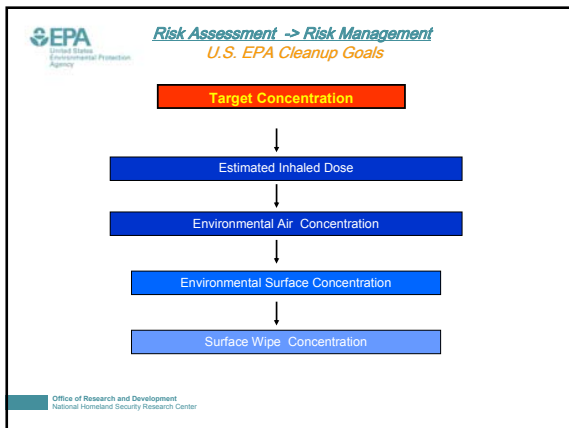
Target Conc. x Intake x Pathogenicity = Risk



Risk-based Goal = Target Conc. = $\frac{\text{Target Risk}}{\text{Intake} \times \text{Pathogenicity}}$

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Target Concentration

How do we derive the target concentrations for biologicals?

What are the minimum data requirements ?

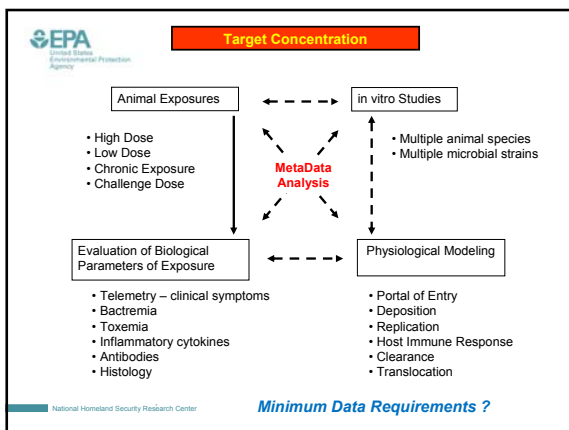
- pathogens vs surrogates
- exposure route
- dosing regimen
- animal model
- correlate of disease

Examples :

Minimum Data Requirements for Registering a Chemical Pesticide, CFR 158

New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible , 21 CFR 601

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Risk Assessment
Clean-up Decision Making Challenges for Biological Agents

- Limited data available on which to base necessary immediate decisions
 - Unique agents
 - Unique exposure durations
 - Unique exposure situations/sites
- No consensus-based microbial risk assessment methodology
 - Little infectivity/dose response data for agents of interest
 - Few transmission models
- Communication and Transfer
 - Clear and understandable guidance

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Target Concentration

Questions:

How do we approach a NOAEL / LOAEL for microorganisms? LOTEL?

What dose - response models do we use and why?

How do we design in vivo and in vitro studies to better inform physiological modeling?

How do we extrapolate animal study data to humans?

How do we account for uncertainty and variability ?

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Risk Management
Considerations Impacting Cleanup

- Risk reduction
- Regulatory mandates
- Long-term effectiveness
- Reduction of hazard through treatment
- Short-term effectiveness
- Implementability
- Cost
- State acceptance
- Community acceptance

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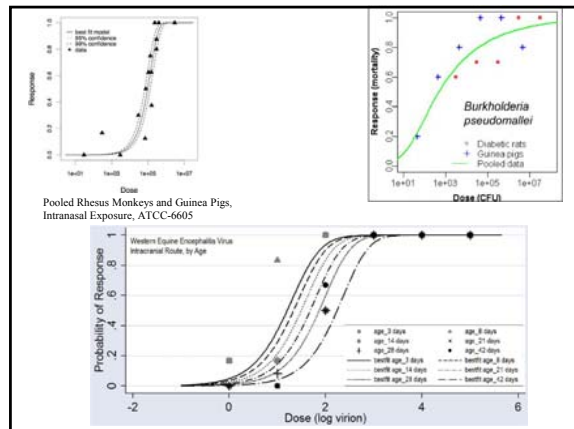
Two Guiding Principles

“Let the Data Speak”
Use of D-R Models that can be
derived from plausible
mechanistic concepts

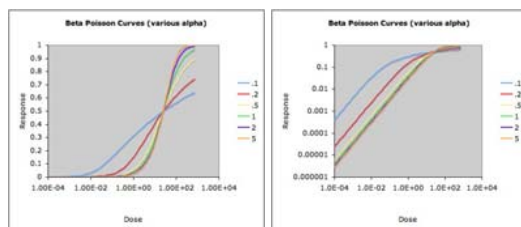
Chuck Haas, Ph.D.

How is uncertainty and variability
addressed in extrapolating dose-
response data (e.g. extrapolating
across host species, exposure levels,
routes of exposure, durations of
exposures, pathogen strains or
species, endpoints, and/or sensitive
populations)?

- Intrinsic maximum likelihood fitting accounts for experimental variability and ascertains if other sources of variability are present
- Parametric uncertainty can be determined
- Tests for pooling between strains, species, hosts, sensitive subpops have all been made (we have examples of all of these in our work)



Frequent Fallacious Understanding of Dose Response Curves



Is it appropriate to group
studies, animal models or host
species, and/or pathogen
strains or species in dose-
response modeling of multiple
data sets?

- Yes if the data justifies it
- Dose metric generally should be ingested/inhaled #
- In progress work - looking at in vivo pathogen dynamics to assess body burden as a metric
 - Future - body burden, AUC, etc. as metrics

Dose Response Models Are
Consistent with Human Outbreak
Data (some examples)

- *Legionella pneumophilla*
- *Salmonella typhimurium*
- *Giardia lamblia*
- *E. coli* O157:H7
- *Cryptosporidium parvum*
- *Bacillus anthracis* (Sverdlovsk)
- SARS

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Physiologically-based Modeling

Sarah Taft, PhD
Threat Consequence Assessment Division
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Cincinnati, OH

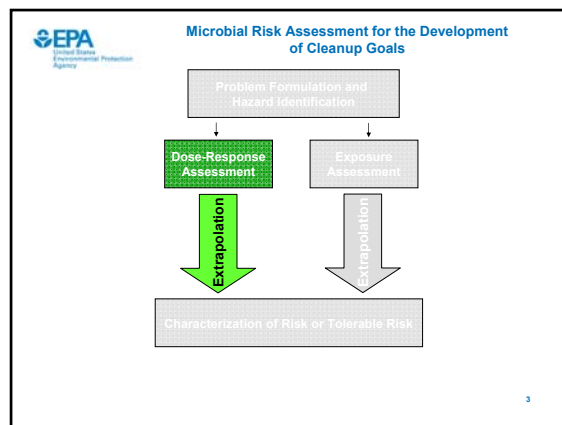
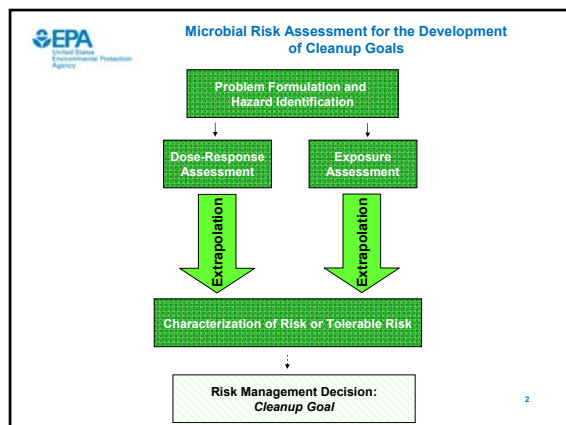


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1. What overall assumptions are necessary for valuable physiological models to predict human consequences?
2. What is the minimum data set required (i.e. what level of detail needs to be modeled for acceptable human predictions (e.g. whole species models, organ-specific models, and/or cellular or toxin activity models)?)

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Microbial Risk Assessment for the Development of Cleanup Goals

Dose-Response Assessment:
Challenges for Biothreat Agents

- Human dose-response data is very limited!
 - Naturally occurring human cases extremely rare
- Un-ethical to perform studies using human volunteers
 - Most of these agents potentially lethal

Dose-Response Assessment

Must rely on animal models to predict human consequences

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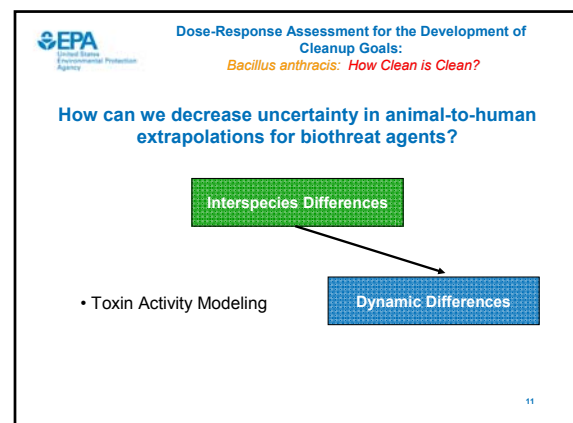
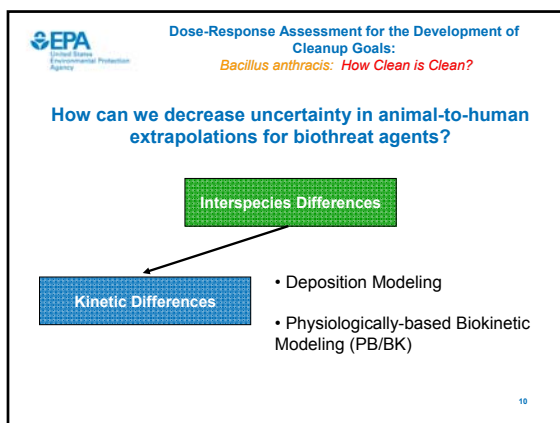
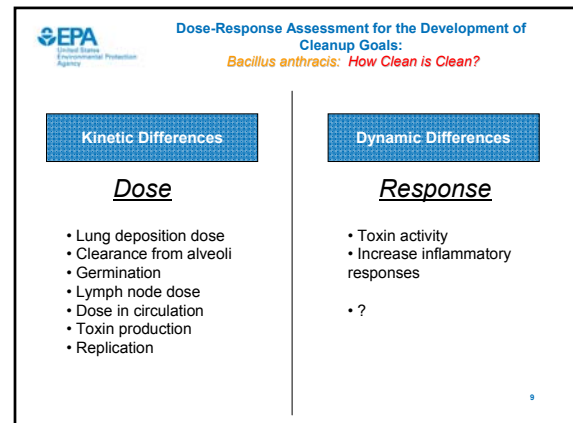
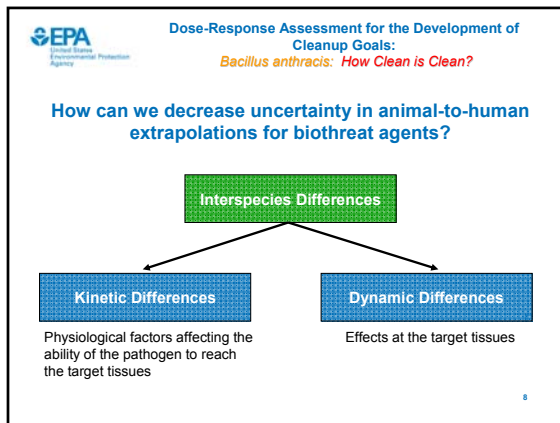
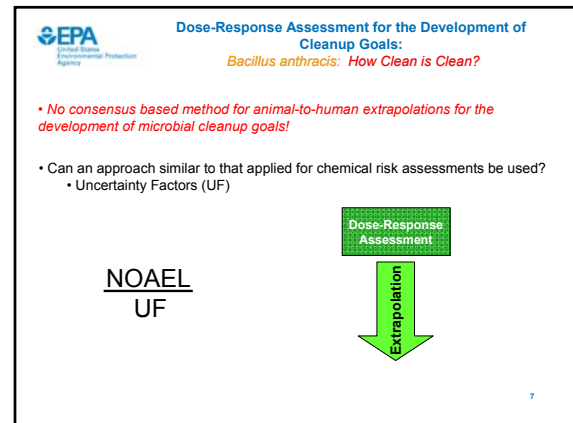
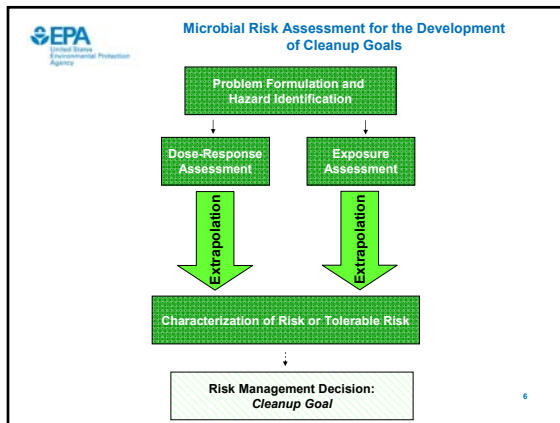
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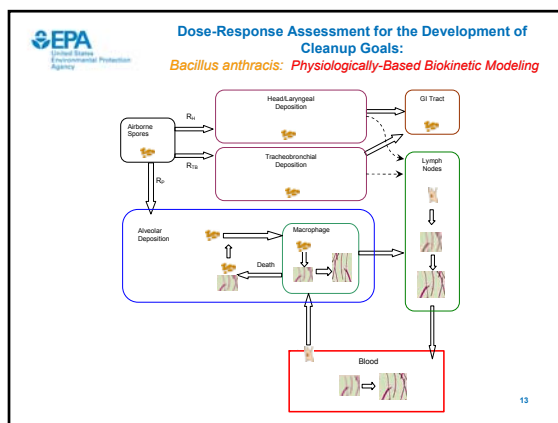
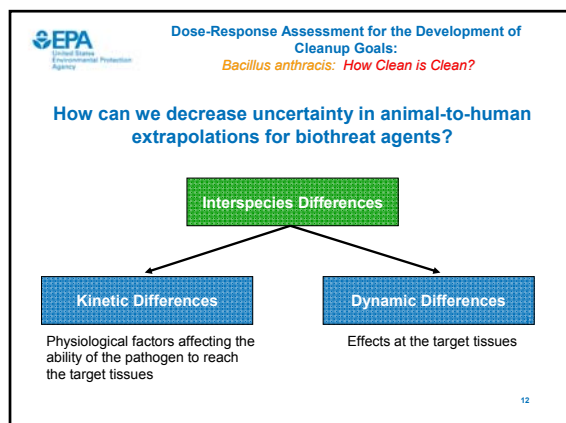
Microbial Risk Assessment for the Development of Cleanup Goals

Dose-Response Assessment:
Animal Models to Predict Human Consequences

- "Best case" scenario = Animal model meets the following criteria:
 - Disease is caused by the same mechanism of pathogenicity
 - Similar physiological and immunological responses to the agent
 - Comparable quantitative relationships between infectivity, morbidity, and mortality

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Mission Needs for Dose Response at FDA-CFSAN's Microbial Risk Assessment Program

April 21, 2009

David, O. Oryang, M.S
DHHS/FDA/CFSAN

DHHS/FDA/CFSAN

Background (1)

- FDA has a long history of managing risks, conducting safety assessments and risk assessments for food additives, chemicals, and microorganisms



1906 – Dining room of “poison squad”:
A direct approach to assessing risk

DHHS/FDA/CFSAN

Background (2)

- CFSAN is moving toward a more risk analysis based approach:
 - Develop and use efficient means to collect, organize, review and share information used in regulatory decisions
 - Prioritize activities because of limited resources

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Microbial Contamination: FDA Centers

Microbial contamination is a source of concern to several FDA Centers

- CFSAN: foods
- CVM: meat, eggs, seafood
- CDRH: medical devices (sutures)
- CBER: blood products and vaccines

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Center for Food Safety and Applied Nutrition (CFSAN)



Mission:
Promoting and protecting public health by ensuring that our food supply is safe, sanitary, wholesome, and honestly labeled, and that cosmetic products are safe and properly labeled

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Risk-based Decisions

Growing responsibilities and new challenges require new tools and approaches

Risk assessment is a tool used by regulatory agencies to support decision making for: import policies, control strategies, inspection programs, tolerance levels, etc.



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Decisions...

- Each day industry and government agencies must make decisions about the safety of foods and food products
 - The public health and economic well-being consequences of "bad" decisions can be substantial
 - Not deciding is not an option

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Informed Decisions...

During the past 15 years there has been a tremendous effort both in the United States and throughout the international food safety community to make decisions that are:

- science-based
- risk-based
- transparent
- consistent

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Risk Assessment

Risk Assessment is one of three components of the risk analysis triad: Assessment, Management, and Communication.

- A process to describe what we know and how certain we are of what we know.
- Answers 4 key questions:
 - What can go wrong?
 - How likely is it to occur?
 - What are the consequences?
 - What factors can influence it?



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Uses for Risk Assessment

- Know where to look**
 - Set priorities/ allocate resources
 - Identify steps along "farm to fork" continuum that are "major contributors" to risk
- Evaluate effectiveness of interventions**
 - Potential or equivalent control measures
 - Proposed standards and criteria
 - Contribution of compliance to risk management
- Inform communication/outreach messages**
 - Determine subpopulations "at increased risk"
 - Assess uncertainty and variability
 - Present objective comparison of alternatives

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Determination of the Dose-Response is part of the Risk Assessment

- Answers 4 key questions:
 - What can go wrong? -
 - Consumption = illness? (Exposure → Infection → Illness)
 - How likely is it to occur?
 - Likelihood/Frequency of adverse effect. f(dose, susc, path., etc)
 - What are the consequences?
 - Severity of adverse effect: illness, death, etc)
 - What factors can influence it?
 - (Pathogen, Host, and Environment factors)

Mathematically Modelled as:

- Exponential
- Beta-Poisson, Log-Normal, Log-Logistic, Extreme-Value
- Weibull-Gamma, Exponential-Gamma

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FDA's Role in Food Safety



- Every day across the country, people eat out, buy groceries, and cook meals for their families. Americans expect that all their food will be safe, and FDA plays a critical role in making sure this is true.
- FDA is responsible for the safety of the vast range of food Americans eat; about 80 percent of all food sold in the United States.
- This includes everything except for meat, poultry, and processed egg products, which are regulated by the U.S. Department of Agriculture (USDA).

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Defining the Challenge

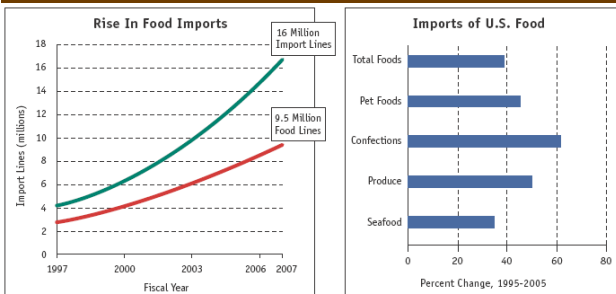
Several factors are imposing increasing demands on FDA's resources.. These are:

- Increases in the volume, variety and complexity of imported foods.
- Shifting demographics.
- Americans are consuming more convenience foods.
- A greater variety of foods are eaten year round. Also, foods that are consumed raw or with minimal processing are often associated with foodborne illness.
- The emergence of new foodborne pathogens.



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Global Food Supply



- The United States trades with over 150 countries/ territories with products coming into over 300 U.S. ports
- It is increasingly important to understand changing consumption patterns by susceptible population.

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Pathogens Newly Associated with Foodborne Illness Since the Mid-1970's

- *Campylobacter jejuni*
- *Cryptosporidium parvum*
- Shiga toxin-producing *E. coli*
- Noroviruses
- *Salmonella* Typhimurium DT104
- *Vibrio cholerae* O139
- *Vibrio parahaemolyticus*
- *Campylobacter fetus*
- *Cyclospora cayentensis*
- *Listeria monocytogenes*
- *Salmonella* Enteritidis
- *Vibrio vulnificus*
- *Yersinia enterocolitica*
- *Enterobacter sakazakii*

- The emergence of new foodborne pathogens requires updated technologies that can detect the presence of new agents in a variety of foods.
- Addressing these emerging hazards requires cooperation among industry, academia, and government to share information, establish testing protocols, and develop dose-response data and relationships.

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Looking Forward:

- **Growth models:** More effective estimates of exposure levels.
- CFSAN focus on acute, as well as **transient/chronic effects**.
- **Susceptible populations** - IRAC working group, Food Forum symposium.
 - Variation in susceptibility within age groups
 - Variation in susceptibility between age groups
 - Variation in fatality to hospitalization ratio
- Increase **accessibility to data**, models and information.
- **Dose-response relations** for new foodborne pathogens. Extrapolate data acquired in animal models to humans.
- **Web based tools** for risk ranking across products and hazards (IRISK)
- **Development of risk prioritization framework** to allocate resources across programs on the basis of risk and other factors.

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FoodRisk.org

The online resource for food safety risk analysis

Welcome to FoodRisk.org

The aim of FoodRisk.org is to assist professionals involved with the many aspects of risk analysis as it pertains to the safety of our food. Thus, we provide data, tutorials, tools, and links to numerous sources of information. In addition to providing resources for the food safety risk analysis professional, we also provide several [consumer-oriented links](#).

For people new to the field, we have a [brief introduction](#) to the field of food safety risk analysis.

Exclusives

Food Safety Alerts
56 FR No. 229 (1991) (Proposed HLEA)
This is the PDF version of the Proposed Federal Register Part III released on November 27, 1991 and preceded the final Federal Register Part II released on January 6, 1995. This document pertains specifically to the Department of Health and Human Services' Food and Drug Administration. All pages were scanned from the original, including text, charts, images and bibliographical references. [read more](#)

FoodSure 2007 Cold Temperature Database
FoodSure, the food safety & quality assurance division of FoodLab, has updated the 1999 Audits International cold temperature data base. In addition to an updated data set, the new data help determine whether changes have occurred in cold temperature storage practices of food both in retail establishments and in consumer homes. [read more](#)

Developing responses to emerging global food safety issues
Abstracts and presentations for 2008 CFS/CFR/IRAC Joint Symposium on Food Safety and Nutrition. [read more](#)

IRAC-sponsored Public Conference: Risk Assessment, Economic Analysis, and Foodborne Illness Regulations Proceedings
Proceedings for 2007 IRAC Public Conference [read more](#)

Research Tools

- Dose-Response Tools
- Exposure Tools
- Modeling and Simulation
- Monitoring & Surveillance
- Hazard Assessment
- Predictive Micro-Tools

iRISK – A web-based Comparative Risk Tool

- Developed by FDA/IFT, and operationalized by RSI.
- Used to compare relative food safety risks across a wide variety of chemical and microbial hazards, foods and processes.
- Key feature: individual users have the ability to securely develop risk models within the program repository and can easily share data and models with colleagues.
- Available thru www.foodrisk.org

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Conclusion

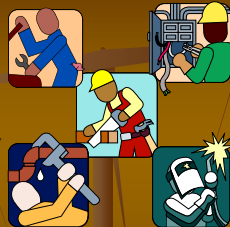
- A key component of MRA is dose-response modeling.
- Need to better define susceptible populations for microbial hazards, and address host susceptibility variation in the dose-responses.
- Development of better process, survival, and growth models → better exposure assessments,

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Risk-based Decisions

Growing responsibilities and new challenges require new tools and approaches

Risk assessment is a tool used by regulatory agencies to support decision making for: import policies, control strategies, inspection programs, tolerance levels, etc.



- Each day industry and government agencies must make decisions about the safety of foods and food products
 - The public health and economic well-being consequences of "bad" decisions can be substantial
 - **Not deciding is not an option**

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To Advance the Field of Food Safety Risk Assessment We must continue to:



- Learn from our experiences
- Develop new ways to address complex food safety issues
- Foster involvement of multi-disciplinary expertise
- Actively participate in international activities

- Improve exposure assessment
- Improve dose-response modeling
- Define and characterize susceptible populations

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Modes of Action in Low-Dose Extrapolation

Laurie Waisel, PhD, Concurrent Technologies Corporation
 Thomas Whalen, PhD, Georgia State University
 Thomas Taylor, MS, PE, Centers for Disease Control
 Murray Cohen, PhD, MPH, CIH, Frontline Healthcare Workers Safety Foundation

EPA-CDC Workshop on Dose-Response Relationships for Microbial Risk Assessment
 Atlanta, Georgia
 April 21-23, 2009

Thanks to Joey Kierman for the artwork!



(The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention)

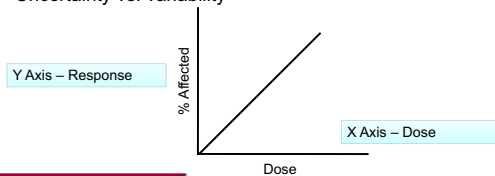
Roadmap

- Modes of Action
- Low Dose-Response Extrapolation Workshop Paper
- Static Models
 - Horizontal Line
 - Vertical Line
 - Diagonal Line
 - Probit
- Dynamic Models
- Variability and Uncertainty
 - Deterministic: Individual Differences (Variability)
 - Stochastic: Lucky Germs (Uncertainty)
 - Stochastic: Unlucky Hosts (Uncertainty)



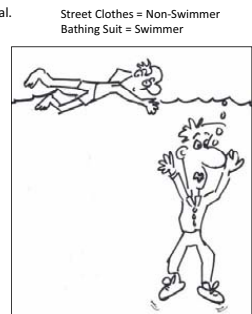
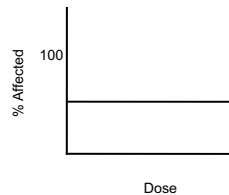
Modes of Action

- Not a mechanism of action
- Dose-response extrapolation curve
- Decision sciences (applied math)
- Static vs. dynamic
- Uncertainty vs. variability



Horizontal Line

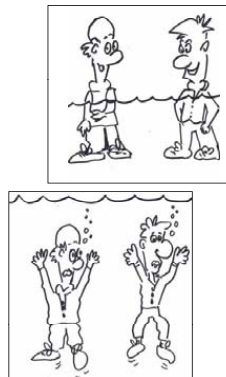
- Susceptible? Yes or No
 - Either you're susceptible or you're not.
 - If you're susceptible, then any dose is lethal.
 - All susceptible individuals affected, regardless of dose.



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Vertical Line

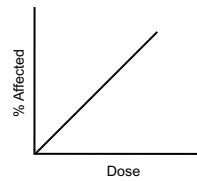
- LD is the same for everyone, so LD01=LD50=LD100.
 - Dose is symbolized by depth of water
 - Resistance is symbolized by height.
 - Everyone has the same resistance.



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Diagonal Line

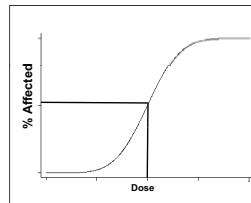
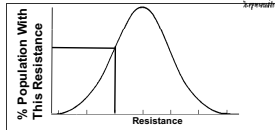
- Risk directly proportional to dose
 - Concept: Number of targets hit is directly proportional to the number of bullets fired
 - Example: Carcinogenic radiation
 - Number of snowballs symbolizes dose of radiation



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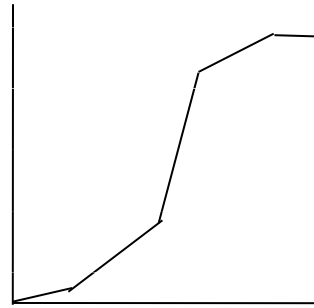
Probit

- Resistance is normally distributed.
- Dose-response relationship follows cumulative normal distribution.
- In this example, resistance is symbolized by height.
- In this example, the proportion of people who will drown in a given depth of water follows the probit (cumulative normal) distribution



Multi-Parameter

- Multiple parameters determine resistance



Dynamic Models

- Time matters.
- Repair and reversibility.
- Can the man bail out water faster than it comes in?
- How long can he keep bailing?



Variability and Uncertainty

- Variability**
 - Dose-response relationship depends on individual differences
 - Deterministic
 - If you have all the information, you know the answer with certainty.
 - Example: diagonal line model
- Uncertainty**
 - Dose-response relationship has an element of chance
 - Stochastic or probabilistic
 - Even if you have all the information, you cannot know the answer with certainty because the answer is partially determined by chance.
 - Example: radioactive decay

The actual dose entering the host may be uncertain depending on the routes of exposure.

Deterministic: Individual Differences

- Dose-response relationship depends on quantifiable individual characteristics of human and microbe.
- Is the virulence of the germ bigger than your ability to resist?
- Whichever one is bigger will win



Stochastic: Lucky Germs

- Each spore has a given probability of germinating (attack rate).
- Lucky spores are the ones that are randomly selected to germinate.
- Lucky germ hits the jackpot at the one-armed bandit.



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Stochastic: Unlucky Hosts

- If exposed to disease, there is a given probability that illness will occur.
- Unlucky hosts are the ones who are exposed and get sick.
- Lucky hosts are the ones who are exposed and do not get sick.
- Unlucky host loses all his money at the one-armed bandit.



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Decision Science

- Make sure who needs to make what actual real-world decision, and why they need to make it.
- Integrate theoretical and empirical, qualitative and quantitative thinking to make that decision as well-informed as possible.
- Decide which model to use.
 - Passes within the error bars of the data?
 - Make biological sense?
- Test selected model against empirical data.



Dose-response: Economics and public policy (or, the value of risk)

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Disclaimers

- The findings and conclusions in this presentation are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention



Policy makers and dose-response

- Dose-response all about risk
 - The “uh-oh” moment
- Models inform about trade offs
- Zero risk, or “perfect” vaccine/ drug, BUT
 - What about cost?
 - Side effects?
 - Practical – can it be achieved?



Policy decisions: Example 1: The anthrax letters: To recommend vaccine or not?

Figure 1: Effect of different duration of anthrax post-exposure prophylaxis + spore survival data

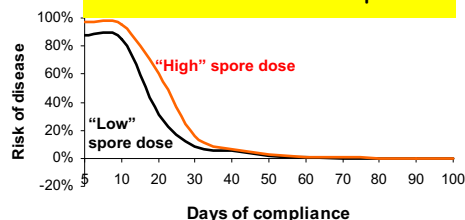


Sources: 1) Henderson et al. J Hyg (Lond). 1956;54:28-36; Fig 2 for Days 5, 10, 20
2) Friedlander et al. J Infect Dis. 1993;167(5):1239-43. Table 1 for day 30

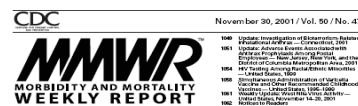


Policy decisions: Example 1: The anthrax letters: To recommend vaccine or not?

Figure 2: Assumed risk of disease by duration of antibiotic compliance



Sources: 1) Henderson et al. J Hyg (Lond). 1956;54:28-36; Fig 2 for Days 5, 10, 20
2) Friedlander et al. J Infect Dis. 1993;167(5):1239-43. Table 1 for day 30



Update: Investigation of Bioterrorism-Related Inhalational Anthrax — Antimicrobial Postexposure Prophylaxis for Anthrax: Adverse Events and Adherence

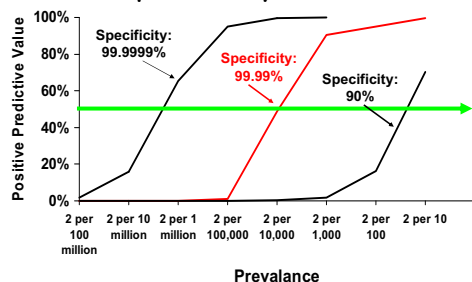
Colin W. Shepard,* Montse Soriano-Gabarro,* Elizabeth R. Zell,* James Hayslett,*

“Overall adherence during 60 days of antimicrobial prophylaxis was poor (44%), . . .”

Emerging Infectious Diseases • Vol. 8, No. 10, October 2002



Sensors and decision making: Specificity* and PPV



Appreciating reality: stem cell transplant cures

Transplant type	Patient	Estimates: cure % MDs	Actual (CI)
Autologous	70	32	44 (30-58)
Allogeneic			
Early	80	62	52 (40-64)
Intermediate	73	42	32 (21-44)
Advanced	80	31	10 (0-23)

Source: Lee et al. JAMA, 2001;285:1034-1038



Smallpox: Risks & benefits of pre-exposure vaccination

Hospital personnel
General populace
Investigation teams

Martin I. Meltzer, Ph.D.
DEISS/NCPDCID/CCID/CDC
(Emerg Infect Dis 2003;9:1363- 1370)

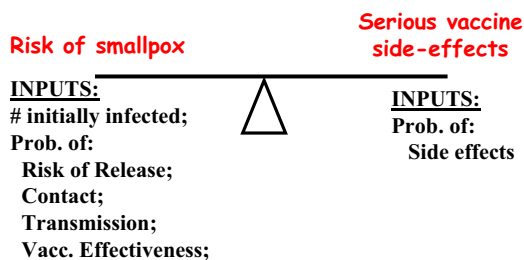


First Example

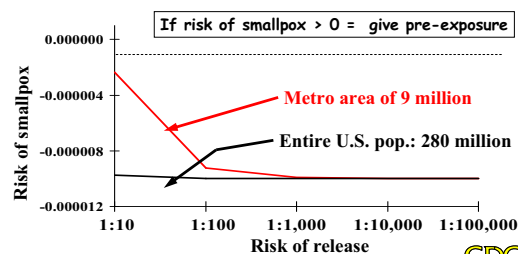
- Response to smallpox as a bioterror weapon
- Dec, 2002 survey: 61% accept smallpox vaccination if "... offered as a precaution . . ."
- Blendon et al. NEJM 2003:348-354.



The balance: Risk-benefits for the INDIVIDUAL



General populace
Risks: Smallpox vs. side-effects
(risk of side effects: 1:100,000
1,000 infected before detection)



Meltzer: Emerg Infect Dis 2003;9:1363- 1370



Utility: First conclusions

- **Utility: not always = produce a number**
 - Concepts as important as data
- **Highlight data deficiencies**
 - How “thin is the ice?”



Utility: Second conclusions

- **Utility: Can we “capture” all the issues?**
 - Use proxies for many items
 - Limit to how many issues can be model
- **Balance: Simplify vs. Simplistic**
- **Utility: Improved when:**
 - One model for a limited set of questions
 - Explain one model doesn’t answer all



Utility: Third conclusions

- **Maximize utility when:**
 - Show changes in results with changes in assumptions
 - Confidence intervals are essential!
- **Maximize utility when:**
 - Describe probabilities
 - Formulas are no good for descriptions!



Dose Response Applications for Vaccines & Therapeutics

 Conrad P. Quinn
NCIRD 

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 **Bacillus anthracis**

How are biomarkers utilized in dose-response modeling of infection and disease?

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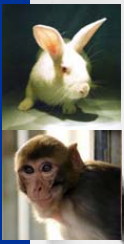
Anthrax Biomarkers

- Exposure $\neq$ Infection $\approx$ Disease
- Exposure
 - May be innocuous
 - Barrier protection
 - May result in a host response
 - Innate, non-specific
 - Specific
- Infection
 - Usually results in a host response
 - Innate, non-specific
 - Specific
- Disease
 - Spore uptake/germination
 - 2^o to integument breach

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Canaries in a Coalmine

21 CFR Parts 314 and 601; the 'animal rule'

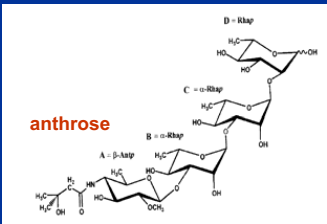
 

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Host Biomarkers for Exposure

- Anti-ATS Responses -

 **BclA**

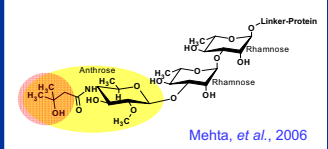
 **anthrose**

Daubenspeck et al., 2004

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Anthrose trisaccharide (ATS)
is antigenic and exposed on the surface of *B. anthracis* Sterne spores

A *B. anthracis*-specific antigenic Region is localized to a defined terminal group of the oligosaccharide

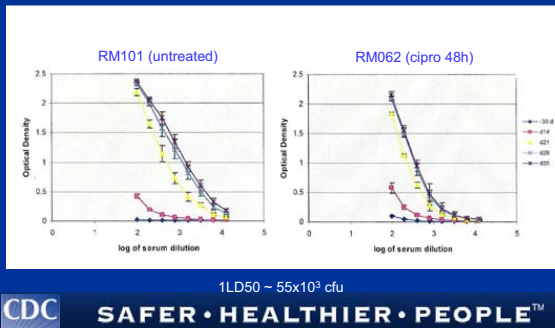


Mehta, et al., 2006

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Host Biomarkers for Exposure

- Anti-ATS Responses -

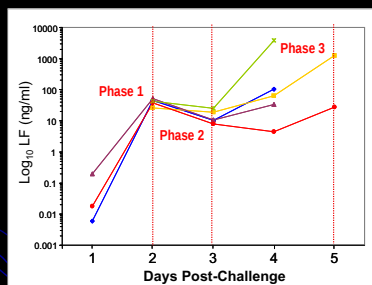


Biomarkers for Infection

- LF Toxemia
 - Specific for anthrax
 - Quantitative LF detection (serum/plasma)
 - Detectable ~18 hr post-exposure
 - T= (18-x) hr post-infection

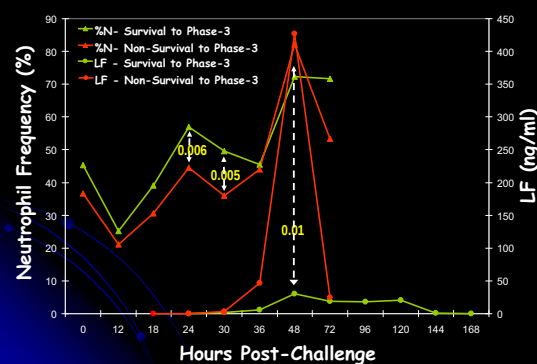
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Triphasic Kinetics of LF Toxemia in rhesus macaques

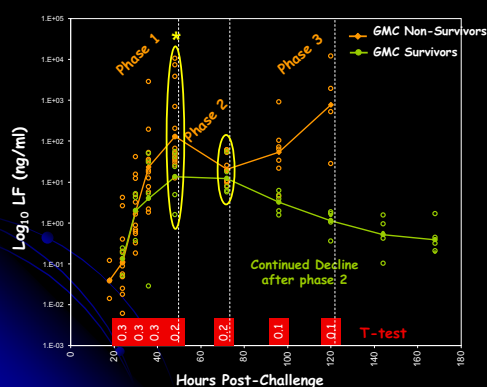


Other biomarkers:
PA detectable later in infection
PGA and bacteremia may be undetectable at end of phase 2

Lethal Factor and Neutrophil Frequency for Death before Phase-3 vs Survival to Phase-3 or Later



LF Levels may predict outcome trajectory



Host Biomarkers for Disease

- Seroconversion to Anti-PA IgG -

- Presentation of PA to host immune system
- Measure of host recognition & response
- Contributing test in diagnosis of 12/22 cases
- Critical contribution in confirmation of 6/22 cases
- Single supporting test in 3/11 cutaneous cases
- 1 CA case did not seroconvert
- No seroconverters other than 22 confirmed cases

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Therapeutics Evaluation

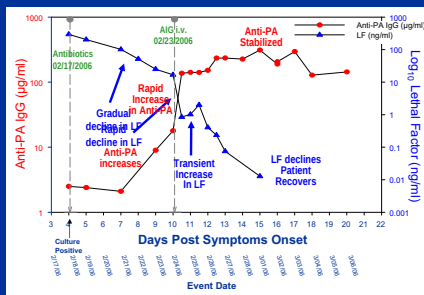
Can dose-response thresholds be estimated for vaccines and therapeutics?



Vaccines & Therapeutics

- Vaccines
 - Field efficacy & immunogenicity studies
 - Non-inferiority vs. "benchmark"
 - Defined correlates of protection
 - Combined measures of surrogacy
- Therapeutics
 - Pharmacokinetics (PK)
 - AUC, C_{max}, V_d, C_L
 - Therapeutic index
 - Ratio of TD50:ED50 (alt. TD1:ED99)
 - Therapeutic window
 - Estimate of effective drug doses within the safety range

Applied Biomarkers for Anthrax



Discussion Points

- Dose response modeling of infection/disease
 - Spore biomarker threshold?
 - Infection threshold?
 - Seroconversion threshold?
- Dose response thresholds for therapeutics
 - Time frame within which the drug is effective?
 - Is there a point of no return?
 - Could treatment exacerbate disease?

Dose-Response Method Comparisons: Bayesian Statistics

EPA-CDC Microbial Risk Assessment (MRA) Workshop
Wednesday, 22 April, 2009

Jade Mitchell-Blackwood
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Bayesian Methods

- Bayesian is a very broad term
 - "Subjectivist" vs. "Frequentist" view
 - Uses Bayes' Theorem to learn from observations
 - Parameters are random variables
- Virtually any approach that uses probability distributions to describe uncertainty in model parameters can be considered Bayesian
- In responding to these points we will try to first note the many options available within a Bayesian framework and then address the approach we have been using as a specific example

1. Is the dose-response statistical method utilized empirical or mechanistic?

- Both types of models can be fit in a Bayesian framework
 - We have generally fit exponential and Beta-Poisson dose response models which can be described as "mechanistic models" (Haas et al. 1999)
- "Mechanistic" because these models are based on biological plausibility:
- Dose is considered random and Poisson distributed in a medium.
 - There is a probability of the host entering a disease state.
 - There is a probability that 1 or more organisms is ingested by the host.
 - There is a survival probability of the organism once it is ingested.

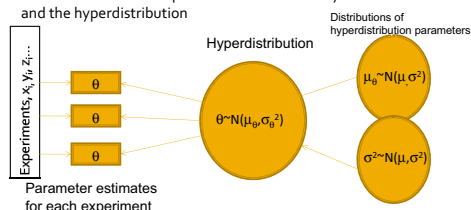
Sometimes called "mechanistically-based empirical" because the parameters are determined empirically from curve fitting to host survival data rather than assessments of individual organism survival rates.

2. Is the method applicable for low-dose extrapolations?

- In general it depends on the model being fit in the Bayesian framework
- Both Exponential and Beta-Poisson are based on sets of assumptions which if true would allow for low dose extrapolations
- Getting adequate low dose data to validate these assumptions is a challenge

3. Can the method accommodate data pooling and/or the use of correction factors?

- Bayesian methods allow for Hierarchical Models
 - Mean parameters generated from individual experiments are drawn from a common distribution (hyperdistribution)
 - Hyperdistribution has hyperparameters
 - Parameters for each experiment are informed by the observable data and the hyperdistribution



An Inter-species variation example

Exponential Dose Response Model

$$P(d) = 1 - e^{-rd}$$

Where:

$P(d)$ = Probability of death

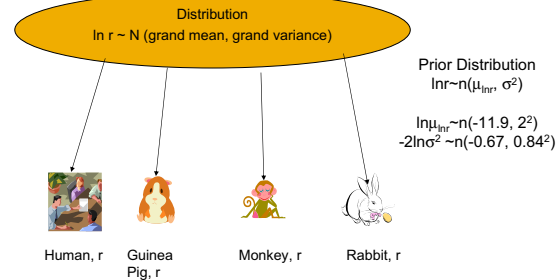
r = pathogen-host survival probability

d = dose of organisms to host

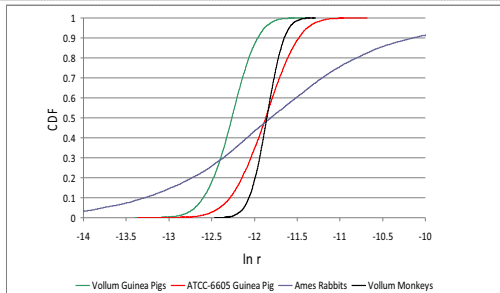
An Inter-Species Variation Example

Organism / Strain Used	Host Species (Reference)	Number of Dose Groups	Minimum Dose	Maximum Dose
<i>Bacillus Anthracis</i> Vollum Strain	Guinea Pig (Altbound, 2002)	6	200	20000000
<i>Bacillus Anthracis</i> ATCC_6605 Strain	Guinea Pig (Altbound, 2002)	6	30	3000000
<i>Bacillus Anthracis</i> Ames Strain	New Zealand White Rabbit (Pitt, 2001)	3	9240000	19110000
<i>Bacillus Anthracis</i> Vollum Strain	Rhesus Monkeys (Druett, H.A., et. al., 1953)	9	70320	398400

An Inter-Species Variation Example



Results of Hierarchical Approach



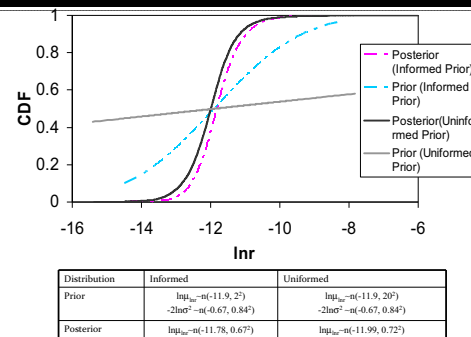
4. How is the calculated dose-response relationship verified and validated?

- Graphical plots of model and data
- Bayesian Information Criterion (BIC)/Deviance Information Criterion (DIC)
- Bayes Factors
 - $\text{Prob}[\text{data}|\text{Model}_1] / \text{Prob}[\text{data}|\text{Model}_2]$
- Cross-validation
 - If sufficient data are available

5. How is model uncertainty adjusted for and communicated to risk managers?

- Parameters are explicitly random variables
- Posterior distribution reflects range of values and likelihoods of different values given both what was known initially and what was learned from the data
- A predictive distribution for unobserved pathogenic agents or species can be generated by integrating over the posterior distribution of the parameters and hyperparameters with measurable uncertainty

Prior vs. Posterior Distributions of the Grand Mean for Unobserved Species



Strengths and weaknesses

- Hierarchical approach allows generalization across experiments and to real world conditions that may not be identical
- Hierarchical approach makes strong assumptions about appropriate distributional forms
 - Large data requirements to validate these assumptions

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