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**Summary Report of the U.S. EPA Workshop on:
*Challenges to Integrating Immunotoxicological and
Microbial Risk Assessment for Susceptible Populations and Life Stages***

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NOTICE

This report was prepared by Versar, Inc., an EPA contractor (Contract No. 68-C-02-061, Task Order No. 136), as a summary of the presentations and discussions held at the U.S. EPA Workshop on *Challenges to Integrating Immunotoxicological and Microbial Risk Assessment for Susceptible Populations and Life Stages* (February 12-13, 2007). 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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The opinions expressed in this document are those of the individual authors and presenters, and do not necessarily represent the policies of the Environmental Protection Agency.

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FOREWORD

Preface to the Workshop Summary

Historically, EPA, and other organizations have focused on individual stressors, whether chemical, physical, or biological, in performing risk assessments. Risk assessors, risk managers and the supporting scientists are well aware that in the “real world” organisms (“receptors”) are usually exposed to multiple stressors simultaneously. Some research has been applied to assessment of multiple chemical exposure and resultant health effects. Other researchers have looked at various sources of sensitivity and susceptibility, including that which results from changes in the immune system throughout the various life stages, from conception to death.

The genesis for this workshop was the recognition that it is difficult to quantify the uncertainty surrounding microbial risks for sensitive groups, populations and lifestages. To complicate risk assessment even further, persons exposed to microbial pathogens are often exposed to chemical stressors, or even immunotoxins. The net effects of these additional stressors, or the effects based on immature immune system, are very real issues to the risk assessors at the EPA. There is also relatively little data on how these factors influence the dose-response to the pathogen(s) of interest. Therefore it was logical to seek out some of the experts in the several fields related to this issue, to determine what the state of the scientific knowledge was in this area.

The EPA Risk Assessment Forum (RAF) convened a workshop of Agency, interagency and invited experts to address the intersection of human life stages, immunotoxicity impacts, and microbial disease susceptibility. The workshop on *Challenges to Integrating Immunotoxicological and Microbial Risk Assessment for Susceptible Populations and Life Stages* was held on February 12-13, 2007 at the Crystal City Marriott in Arlington, Virginia. A wrap-up session was held on March 20, 2007 in Washington, D.C., to identify lessons learned and next steps.

There were 6 invited experts who spoke on the state of the science and facilitated plenary discussions and break out groups; the group was made up of immunotoxicologists, microbiologists, and experts on lifestage susceptibility (in particular children). The experts also wrote short papers on the state of the science in the 3 areas being explored, and these papers were the basis for the presentations, both of which are included in this report. The experts, with participants from several federal agencies, explored the common factors which need to be considered in addressing sensitive or susceptible life stages for both toxicant implications to immunity and altered microbial disease risk outcomes. The workshop participants also considered how chemically-induced immunosuppression and/or the response of the immature immune system may affect susceptibility to infection from common microbial pathogens (the primary focus is limited to early life stages for practical reasons).

The workshop was deemed highly successful by those who planned it, as well as the attendees. Much of the success was by virtue of the exchange of information between scientists of such different fields on related, integrated topics. Participants learned about research findings and

potential data sources, but were simultaneously disappointed by the limited information available to address the key charge questions about quantifying hazard and risk. Some possible short- and long-term research possibilities were explored in the wrap-up session. Further discussions, within the research and programmatic organizations in the government, and in professional circles, are needed to explore the research needs that were identified.

1.0 INTRODUCTION

1.1 Workshop Purpose

The U.S. Environmental Protection Agency's (EPA) Workshop on *Challenges to Integrating Immunotoxicological and Microbial Risk Assessment for Susceptible Populations and Life Stages* was held on February 12-13, 2007 at the Crystal City Marriott in Arlington, Virginia. This two-day workshop was sponsored by EPA's Risk Assessment Forum (RAF) and was organized by Versar, Inc. The EPA's RAF is exploring the common factors considered in addressing sensitive or susceptible populations for both toxicant and microbial risks. This question covers several disciplinary sciences, including immunotoxicology, microbiology, toxicology, and sciences relating to different life stages, such as pediatrics. The objectives of the workshop were to: (1) consider means of integrating the common factors into a risk assessment and (2) determine the next steps and research needs to attain this goal.

1.2 Workshop Participants

A group of nine experts was invited to attend the workshop to assist the Agency with meeting their objectives. The experts, selected by Versar from academia, consulting, and industry, were invited based on their demonstrated experience and scientific expertise in the following three areas: (1) toxicology or multidisciplinary science with expertise in risk assessment of sensitive age groups, e.g., children, aging adults, pregnant females; (2) microbiology with experience in risk assessment; and (3) immunotoxicology with expertise in immune function and stressors, including microbes, and their effect on susceptibility to infection and disease manifestation. The role of the invited experts was to prepare papers and presentations on the "state-of-science" of the various disciplines and facilitate/participate in targeted disciplinary discussions. The "state-of-the science" papers were prepared and distributed to all registered participants prior to the workshop. In addition to the expert participants, more than 50 scientists from EPA and other federal agencies attended the workshop. The complete list of workshop participants is presented in Appendix A. Biographical sketches of the nine expert participants are presented in Appendix B.

1.3 Agenda

The workshop agenda is presented in Appendix C. The agenda was developed by the organizers to include presentations on the "state-of-the-science" followed by discussions on the factors resulting in susceptible groups in populations or lifestages, and issues in quantifying immunotoxicological parameters for risk assessment and microbial dose-response estimation. During scheduled discussion sessions, participants discussed key factors and metrics to address risks faced by susceptible groups, as well as risk assessment implications, applications, research needs, and science-policy issues. A series of charge questions were developed to guide the discussions (Appendix D).

The two-day workshop began with opening remarks, including introductions and a presentation on the purpose and goals of the workshop by EPA. It was followed by EPA's

keynote address, which provided participants with the scientific background and scope for the workshop. The remainder of the first day included presentations by three of the expert participants on susceptibility, immunological end points, and quantitative microbial risk assessment methods. Each presentation was followed by a question and answer period, as well as disciplinary discussions. The first day concluded with a plenary discussion on the goals of the workshop and a poster session.

The second day of the workshop started with a brief summary of the presentations and discussions from day one, as well as a review of the charge for a discussion session. Because of inclement weather, the proceedings of the second day were shortened and included only one plenary discussion session (originally, two breakout sessions had been planned – immunotoxicants and microbial pathogens) that addressed the charge questions, followed by discussion of implications, applications, and next steps. The discussions scheduled for the third day were cancelled because of the weather and were held via teleconference on March 20, 2007. The teleconference, for EPA participants only, included a summary of the high points of the workshop, and discussion of EPA's priority categories for data collection (e.g., new research, available databases), partnering, as well as short- and long-term goals and priorities.

1.4 Organization of Workshop Summary Report

This report provides summaries of the opening remarks, presentations, and discussions from the workshop. Additional information is provided in the appendices.

- Section 2 of this report summarizes the opening remarks and presentations by the expert participants.
- Section 3 summarizes the discussions from Day 1 and Day 2, as well as the teleconference for EPA participants only.
- The appendices to the report include the written and visual materials from the workshop including a list of participants, biographical sketches of expert participants, agenda, charge questions, presentation materials/slides, state-of-the-science papers, flip charts produced during the workshop, and meeting notes from the Day 1 and Day 2 discussions.

2.0 SUMMARY OF OPENING REMARKS/PRESENTATIONS

The following sections summarize the workshop's opening remarks, which include the welcome and introductions, purpose and goals, and keynote presentation on the background and scope of the workshop. Also summarized are the presentations for the three disciplinary areas of susceptibility, immunotoxicology and microbial risk assessment, and a presentation summarizing the overarching issues to be addressed during the workshop discussions. Each of the presentations are provided in Appendix E. The three "state-of-the-science" papers, upon which the three disciplinary presentations were based, are provided in Appendix F.

2.1 Welcome and Introductions

Mr. Gary Bangs, EPA RAF, welcomed the participants and provided a review of the agenda for the workshop, as well as logistical information. He then asked speakers and invited experts to introduce themselves.

Following the introductions, Dr. William H. Benson, EPA Office of the Science Advisor, addressed the workshop participants. He thanked the RAF for putting together a workshop to discuss the challenges associated with chemical and pathogenic stressors. He stated that addressing these challenges would necessitate bringing together people of several different disciplines and encouraging collaboration between public and private sectors.

2.2 Workshop Purpose and Goals

Dr. Stephen Schaub, EPA Office of Water/Office of Science and Technology, made a presentation outlining the purpose and goals of the workshop. He noted that, in 2005, the RAF initiated communication between the Microbial workgroup and the Immunotoxicology workgroup to identify areas for potential cooperation. The Office of Children's Health Protection (OCHP) was also brought into the effort to help address issues associated with life stages and susceptible populations. The goals of the workshop were to discuss the most current scientific information on immunity factors for immunotoxicants and microbial pathogens, and to examine ways in which the workgroups can work together. Dr. Schaub noted that a multidisciplinary approach to dose response characterization for susceptible populations and life stages includes determining what immunological components are of concern and how they interact, and what the data needs are for tools and models. Workshop participants can also consider needs for immunotoxicology and microbial risk assessment guidance and suggest next steps for research and guidance.

Dr. Schaub also addressed specific issues to be discussed during the workshop, which included background discussion on the "state-of-the-science" disciplinary contributions in immunity/dose response for use in human health risk assessments and integrating the disciplinary work to develop or improve risk assessment tools and methods. Experts were invited to discuss their discipline's role in human health risk assessment, and potential modification to dose-response or hazard characterization practices, as well as the effects of each discipline on predicted outcomes and uncertainties. Discussions would include prioritization of parameters or factors to best protect both individuals and public health.

2.3 Background and Scope for Workshop

Dr. Robert Luebke, EPA ORD/National Health and Environmental Effects Research Laboratory (NHEERL), provided the keynote presentation. In his presentation, entitled “Integrating Life Stage Susceptibility into Immunotoxicity and Microbial Risk Assessment,” Dr. Luebke discussed regulatory issues and the current state of risk assessment practice and immunotoxicological hazard identification.

Regulatory mandates exist for taking life stages and susceptibility into consideration when performing risk assessments. These include the Food Quality Protection Act, the Safe Drinking Water Act, and Executive Order No. 13045 (“Protection of Children From Environmental Health Risks and Safety Risks”). The Agency is interested in the use of life stages in risk assessment, with an emphasis on early life stages. Research goals include addressing age (lifestage) at the time of exposure as a variable in determining short- and long-term outcomes of exposure. There are a number of Agency-related initiatives that address life stages and susceptibility. For example, the ILSI/HESI Technical Panel on Agricultural Chemical Safety Assessment is working with Agency and industry experts to develop consensus between government and industry on how best to perform chemical safety assessments for fetal and early life populations. The panel has proposed a two-generation reproduction testing protocol that includes an “enhanced” F1 (first filial generation) component to evaluate developmental immunotoxicity and developmental neurotoxicity endpoints. The Microbial Contaminant Candidate List (CCL) Workgroup takes into consideration life stages and susceptible groups in the development of screening criteria.

Regarding the current state of risk assessment practice, Dr. Luebke stated that microbial and immunotoxicological risk assessors are working independently. Microbial risk assessors have already incorporated life stages into their assessments but have not yet developed approaches to account for potential suppressive effects of immunotoxicants. Immunotoxicity testing guidelines rely on adult exposure studies; this practice may be better suited to protecting the general population instead of age extremes, and it is not clear that application of uncertainty factors will always provide adequate protection for the most susceptible groups.

Dr. Luebke described the basics of immunology. He noted that there are two broad classifications of immune responses, innate and adaptive. Innate responses are triggered by specialized receptors that recognize components shared by many genera of microorganisms. Adaptive responses, on the other hand, are responses to antigens, which are typically unique to small groups of organisms. There are almost an unlimited number of antigens to which the adaptive response can respond. Lymphocytes are the main effectors of adaptive responses, either as sources of proteins or as direct effectors. There is also a memory response that provides a very rapid response the next time an antigen enters the system. The adaptive response process involves recognition of a foreign antigen followed by antigen processing and presentation; this triggers a complex series of events that work properly to ensure adequate immune function. The main players in the adaptive immune response are B and T lymphocytes, which perform different tasks.

The best case scenario following exposure to a chemical is that immune function is not affected, either because the chemical itself is not immunotoxic or because the dose is too low to cause an effect. However, another outcome of chemical exposure is immunosuppression, which increases susceptibility to infection. Exposure may also trigger inappropriate responses to antigens, which can take the form of autoimmunity or allergies. Dr. Luebke noted that sometimes the term “chemical AIDS” is used to refer to the effects of immunosuppressants, but this terminology is not appropriate. Individuals with Acquired Immunodeficiency Syndrome, and other forms of severe immunosuppression, are likely to develop infections with opportunistic organisms, rather than with commonplace pathogens known to cause increased rates of infection in individuals with mild to moderate levels of immunosuppression. Cases of chemical-induced immunosuppression will normally resolve with time after exposure ceases, unless exposure destroys the bone marrow.

Resistance to infection depends on a number of host factors including age, gender (in pregnancy, females can be more susceptible), genotype, nutritional status, life style choices, and life events. Microbial virulence factors may influence the outcome of infection regardless of host immunocompetence, by allowing microbes to evade destruction by the host. Age alone, in the absence of chemical exposure, is an important factor in the outcome of infections. For example, neutrophils provide a first line of defense against bacterial infections. In newborns, the level of neutrophil production is quite low and may not keep up with infection. Neutrophils from newborns also have only one third to half the concentration of bacteriocidal enzyme that adults have, thus reducing the killing efficiency at the single cell level. Newborns initially have adequate antibody-mediated protection to organisms that their mothers have immunity to, due to transfer of maternal antibody across the placenta. However, antibody levels decreases to 50% of adult levels by 7-12 months, due to catabolism of maternally derived antibodies and adult levels of antibody production are not attained for some time. Immune function is also less in the aged population, due to increased cell loss and reduced cell function.

Currently, research on immunotoxicological hazard identification is conducted by some government, industry, contract, and academic laboratories. EPA has published harmonized testing guidelines for immunosuppression (EPA Health Effects Test Guidelines 870.7800) that call for exposure of adult animals to the test article for 28 days and evaluation of antibody production in response to immunization. If immunosuppression is detected, phenotypic analysis may be performed, and, on a case-by-case basis, mature killer cells can be examined. It is not clear whether testing only in adult animals is adequate. He noted that data from studies that evaluated adult and developmental exposure to lead, diethylstilbestrol, diazepam, dioxin or tributyltin oxide, all well characterized immunotoxicants, were examined to determine if similar results were obtained following adult or developmental exposure. It was found that adult testing would usually detect potential immunotoxicity, but may underestimated the LOAEL for immunotoxicity or the persistence of effects following developmental exposure. In the young, lower doses of immunotoxins can cause long-lasting effects, even though the same doses would not affect immune function in adults. In order to extrapolate results from rodent studies to humans, it is necessary to consider differences in the rates of immune system maturation in humans and rodents, relative to birth. Various exposure scenarios have been proposed for animal models, although many immunotoxicologists agree that exposure during gestation, weaning and

early adulthood has the best chance to detect developmental immunotoxicants. Dr. Luebke noted that while developmental immunotoxicity testing continues to evolve, very limited data are available for the elderly population, another group that is characterized by age-related immunosuppression. Nevertheless, available human data on school children, the elderly (>65), and stressed populations suggest that increased rates of infection correlate with extrinsic factors (stress, chemicals) that cause mild to moderate immunosuppression.

Dr. Luebke discussed some issues to be considered when attempting to integrate life stage susceptibility to immunosuppression into microbial risk assessment. The first step is to identify the population to be protected. Because there are background rates of infection in otherwise healthy people, detecting small increases in common infections is very difficult at the population level, making case-finding difficult. In addition, it is important to define who will be defined as the “most sensitive subpopulation” that needs protection. There is also the question of how immunotoxicological data can be used to improve microbial risk assessment. In incorporating life stages in microbial risk assessment and immunotoxicity models, it is important to evaluate whether current models have adequate sensitivity to capture small changes at the population level. It is also critical to determine whether the default microbial risk assessment assumptions are adequate to predict changes in life stage susceptibility and whether immunotoxicological data can be incorporated into current microbial models. Successful incorporation of animal-derived life stage data into immunotoxicity risk assessment will require the ability to extrapolate animal data, that typically describes infection severity, to potential disease incidence and severity in humans. It is also worth noting that animal data are derived under far more carefully controlled conditions than most human studies.

To conclude his presentation, Dr. Luebke presented two options for conducting risk assessments of chemical and pathogenic stressors. One option is to include immunology data in models used in microbial risk assessment, to account for potential sensitivity to environmental immunotoxicants. The second option is to have immunological risk assessors run a model that includes microbial susceptibility data. Ideally, everything will be brought together in a way that protects the general population as well as susceptible life stages and populations. Dr. Luebke noted that, hopefully, this can be performed using means other than additional uncertainty factors.

Questions/Answers and Discussion

A workshop attendee asked Dr. Luebke what is considered an aged population in humans. Dr. Luebke indicated that the values he has seen in the literature indicate that the cutoff is about 60-65 years old. It was also noted that the older population is heterogeneous and more likely to have infectious disease in the absence of chemical exposure.

Another attendee stated that the data comparing young adults to animals, in terms of parasite immunity, are very interesting. He noted that the aged test animals were not exposed to environmental stressors because they grew in a sterile environment. He asked whether there was a better approach than using animals that atrophy in the absence of any environmental challenges. It was noted that there had been studies done on deer mice that live in landfills, and that scientists had had trouble infecting them. In a laboratory setting, it would be difficult to

replicate the conditions. Some studies had been done on squirrels that showed differences between wild and laboratory specimens.

It was stated that most studies used a defined set of microbes for studying infection. It was questioned whether using a wider spectrum of organisms would be useful. Dr. Luebke noted that *Listeria*, a commonly used bacterium, is typically chosen because the host responses that mediate resistance to the organism are well characterized (it requires activated T-cells and phagocytic cells). Because the mechanism is known, scientists can pinpoint what part of the immunoresponse is being affected. Using other organisms might make it difficult to reach such conclusions.

In talking about differences between animals and humans, one attendee noted that it is important to recognize that organs, as well as components of the immune system, don't mature at the same rates. In extrapolating results from studies, this needs to be taken into account.

A workshop attendee stated that the focus of the guidelines right now is on immunosuppression and asked how the guidelines address the fact that autoreactive immune cells may be produced. It was noted that the guidelines do not directly address issues such as autoreactive immune cells being produced. Currently, the only functional assay being done is antibody response to T-cells. However, any change in immune system following exposure to a chemical may be an indication that the immune system had been affected. At this point, fewer models are available to address autoimmunity than immunosuppression.

2.4 Susceptibility as a Function of Life Stage

In a presentation on susceptibility, Dr. George Daston, Proctor & Gamble, discussed the children's health risk assessment framework, critical periods of development, and the interactions of genotype, exposure, and host factors. He indicated that development is a uniquely susceptible time as the example of thalidomide-induced malformations indicates. The effects resulting from exposure during development are both qualitatively and quantitatively different from effects seen as a result of exposure at other life stages. It is important to evaluate effects around the time of birth and just after birth since there is indication that exposures during those life stages are different. The Barker hypothesis started with an evaluation of men in their middle age who were suffering from coronary heart disease but had none of the obvious risk factors. Barker looked at the men's medical history and found an excellent correlation between birth weight and cardiovascular disease.

Dr. Daston stated that, from a regulatory context, developmental risk has been a concern for a long time. The developmental reference dose (RfD_{DT}) has been in place since 1989. However, children's risk assessment got formal recognition with the publication in 1993 of "Pesticides in the Diets of Infants and Children" by the National Research Council (NRC). This document emphasizes that exposures in kids might be unusual compared to adults because of behavioral and pharmacokinetic reasons. Following this document, an executive order was published during the Clinton administration which stated that, while therapeutic agents were being prescribed to the entire population, some of that population was not included in testing.

The Food Quality Protection Act (FQPA), which was adopted in 1996, requires that a child-specific additional safety factor be used if the data are not sufficient to adequately assess children's health. EPA now has an Office of Children's Health Protection to help address child-specific issues.

Dr. Daston noted that many changes take place very rapidly during development, which makes children susceptible. In the first weeks of development, cell proliferation and cell differentiation occur, and a body plan is established. Over a period of time, cells go through functional maturation. Any perturbations during this time can be very significant and lead to permanent alterations. It is very difficult for the organism to recover from these perturbations. Usually, it is during or just prior to the appearance of a given organ system's structure in the embryo (before its maturation) that the system is most susceptible to perturbation. For each system, there are unique periods of susceptibility or critical periods, depending on when the system develops. For the immune system, there is a period of functional maturation that is very important. For the immune system, the period of maximum susceptibility extends beyond the critical period for structure development. For organ systems with a large postnatal maturation component, it is possible that persistent changes can occur for a long period of time.

Dr. Daston stated that there are some mechanistic and exposure considerations that are taken into account in children's health risk assessment. For example, there is the question of whether there are mechanisms of toxicity that are unique to childhood. Even though our level of understating of toxicity is increasing, it has not been confirmed that there are mechanisms that are exclusive to a particular life stage. However, it is clear that outcomes can be radically different depending on life stage. Exposure to retinoic acid receptor ligands, for example, produces different outcomes depending on whether exposure occurs early during pregnancy, childhood, or adulthood. Children's risk assessments also consider whether there are behaviors peculiar to children that make exposure by certain routes or media a special concern. Examples of behaviors to consider include: breastfeeding, mouthing, a narrow food selection, and a breathing zone close to the floor.

Dr. Daston further noted that the framework for children's health risk assessment includes three phases: problem formulation, analysis, and risk characterization. The first phase, problem formulation, involves defining the objective of the risk assessment. Issues to consider in this phase are exposure, biological effects, and host factors. Host factors can be complex in children's risk assessment and might include variability in nutritional variability, genotype, etc. Host factors such as genes and environmental exposures are important to recognize. Susceptibility is a combination of genotype and interaction with the environment. When looking at developmental outcomes, the available literature on causes of birth defects indicate that only 3% of birth defects are exclusively caused by toxicants. Similarly, a small percentage of birth defects can be attributed to a single gene defect. The available literature indicate that 28% of birth defects are due to genetics, based on findings of familial patterns that suggest some kind of genetic component, but this does not necessarily exclude environmental components. About 43% of birth defects are due to unknown causes; most likely these defects are due to interactions of several factors. An important host factor that may influence susceptibility to microbial infection is passive immunity obtained from the mother. There are antibodies expressed in

human milk that reduce enteric infection. These antibodies might even have persistent effects on the immune system.

The analysis phase of the children's health risk assessment framework includes development of a conceptual model, characterization of life-stage specific exposure, and evaluation of potential for life-stage specific health effects. In the analysis phase, issues such as availability of quantitative data, ranking of life stages by exposure, stage specific sensitivity and outcomes, and stage specific kinetic and dynamic considerations need to be examined. A decision also needs to be made about whether further assessment is needed. For lead toxicity, for example, life-stage specific considerations include playing in lead-contaminated play areas, greater exposure to dust from lead-based paint, and possibly pica.

The life-stage specific risk characterization phase of the framework is the culmination of the preceding steps. The life-stage specific assessment is an honest account of what is known and what is not known, along with discussion of uncertainty, to help the risk manager. It may contain a justification for uncertainty factors and quantitative assessments.

Dr. Daston stated that immune system development also needs to be considered in children's risk assessment. The Th1/Th2 model of immune development indicates that as lymphocytes mature, they have the potential to ward off either infectious agents or allergens. In the absence of infectious agents, lymphocytes follow the allergens pathway. Children who grow up in environments where there is more exposure to pathogens have a lower rate of asthma. This raises the question of whether an overly rigorous microbial risk policy could have unintended consequences on the prevalence of allergy.

In concluding his presentation, Dr. Daston noted that children's risk assessors need to consider the unique outcomes from developmental exposures, the unusual exposure scenarios of children, and host factors, such as passive immunity, that are specific to life stage.

Questions/Answers and Discussion

In response to a question from an audience member, Dr. Daston indicated that the areas of fetal abnormality and prenatal endotoxin exposure have not been well studied. There are studies involving dosing pregnant rodents, in which an induction of metallothionein is observed. Other studies suggest that lipopolysaccharide (LPS) may be developmentally beneficial.

Another audience member noted that it is important to recognize that development continues beyond the date of birth. For example, air pollution can influence pulmonary growth, such that people in high air-pollution areas have different lungs. It was also noted that exposure to sunburns prior to adolescence may make people more susceptible to melanoma. There is lack of information regarding effects of exposure during puberty.

2.5 Immunotoxicology: Using Immunological End Points to Predict Adverse Effects

Dr. Stephen Pruett, Louisiana State University Health Sciences Center, gave a presentation focusing on predicting adverse effects using immunological endpoints. In the presentation, he provided background information, specifically noting that very few, if any, large scale studies have been conducted in which immune responses in human subjects have been compared quantitatively with changes in host resistance in the same population. Despite this, Dr. Pruett noted that there have been several studies relating chemical exposure to infectious diseases and that results obtained to date suggest that a goal to evaluate immune parameters may be feasible. He also noted that numerous animal studies have been done and the results may serve as a guide for human studies in the future.

Several examples were presented from studies using immunological endpoints to predict adverse health effects. Those studies had the following findings:

- A correlation exists between persistent organic pollutant (POP) exposure from a high marine animal diet and otitis media in breastfed infants.
- A correlation exists between small changes in granulocytes and lymphocyte numbers, and the incidence of HSV-induced cold sore incidents.
- For elderly caregivers of dementia patients, there is a decrease in immune response due to chronic stress, as well as decreased immune response to vaccines and increased incidence of respiratory infections.
- There is an inverse correlation between cortisol levels in the serum and CD8+ T-cells.
- There is a direct correlation to upper respiratory infection following a negative life event.
- Immune measures and responses to vaccines decrease with age.

Dr. Pruett stated it was important to determine how changes in the immune system correspond to changes in resistance to infection. To illustrate this, he presented supporting data showing the correlation between host resistance and immune parameters from chemicals tested by the National Toxicology Program (NTP). Taken together, the immune parameters were highly correlated with changes in host resistance to infectious agent or tumor cell challenge. However, no one test was highly correlated suggesting that one individual immune parameter is not likely to provide a clear quantitative picture of resistance to infection.

Dr. Pruett described possible approaches and problems for human risk assessment. The ideal approach would involve the direct evaluation of immunological parameters, incidence and severity of infections, and exposure to immunotoxicants in a single population. However, the data presently available are insufficient for such evaluation. Studies involving large populations are needed, and it is unclear if sufficient numbers of individuals exposed to the toxicant of interest could be found. The logistics would be complex, and at least one of the factors

previously described in the approach is missing in almost every experiment. Dr. Pruett suggested several alternative approaches to determine risk:

- A parallelogram model was developed to potentially validate animal data by interspecies extrapolation. This model addresses known immune function changes following *in vitro* exposure in both humans and animals, and predicts the susceptibility of infection in humans based on the known animal data.
- A quantitative predictive model based on animal studies measures the suppression of immune parameters for mouse and human cells following *in vitro* exposure to a toxicant. He suggested devising an algorithm relating the quantitative effects on mouse and human cells, measuring the suppression of resistance to infection in mice, and substituting the amount into the algorithm to estimate the value in humans. He noted several problems with this approach, which included the need for human and mouse dose response data, decreased reliability in interspecies extrapolations, the inability of *in vitro* studies to detect immunotoxic metabolites, and the relationship between morbidity and mortality (animal endpoint) and incidence (human endpoint).
- Host resistance data could be obtained using a panel of pathogens and tumor cells. A safety factor could be applied and data from animals could be used to estimate the minimum dosage of toxicant that will decrease host resistance.
- A mathematical model that uses NTP data in conjunction with multivariate methods or neural networks can be used to predict changes in host resistance in mice. Comparable immunological parameters could be measured using human blood samples from exposed individuals.

Dr. Pruett concluded his presentation by addressing how to deal with susceptible groups. Currently, a 10-fold uncertainty factor is used in risk assessments for these groups. Another option could be to use an adjustment factor based on human data showing decreased efficacy of vaccine or increased rate of infection in susceptible groups. A final option presented was to build a comprehensive model incorporating the following: age dependent changes in resistance of particular microbes, quantity and duration of exposure to toxicants, measured changes in immunological parameters, and the difference in virulence of microbes and the amount of typical exposure.

Questions/Answers and Discussion

A question was asked as to how genomics would fit into linking human components with mice data. Dr. Pruett responded that genomics have been demonstrated to be useful for mechanistic risk assessment, but its use in microbial/immunotoxicological risk assessment is less developed. However, using the parallelogram approach described in the presentation, the presence of a marker could allow scientists to test the validity of the assumed safe level. If genomics were used even in hazard identification, one would question whether to use tissue from an animal that has just been exposed, or from one that has been exposed and immunized.

Ideally, the broader-scale results of genomics might be usable, rather than selecting any particular marker.

A participant then asked Dr. Pruett if he knew of any types of infections that affect children versus the elderly and if there were any data that demonstrated that those infections followed a specific immunological profile. Dr. Pruett believed there were probably studies available, but he was not certain. He stated that early indicators of immune senescence included T-lymphocytes and phagocytic cell function. As far as types of infection, the infection most common in elderly is community-acquired pneumonia. Dr. Pruett didn't know if there was any way of linking the indicator and the infection.

A question was asked about the level of confidence given to models like the physiologically based pharmacokinetic (PBPK) model. Dr. Pruett indicated that he was not aware of the PBPK model being used for immunotoxicological modeling purposes.

A participant noted that Dr. Pruett talked about several different dose methods (area under the curve, mg/kg dose, and mg/m²) that can be used to form the relationship between dose models. He asked Dr. Pruett if the relationships were only evident after much studying. Dr. Pruett stated that the relationships are not predictable. For example, the conversion of data from mg/kg to mg/m² is not easily predictable. He noted that to protect human health, he would lean to the more conservative side with the dose models.

A question was posed to the participants as to whether the idea of taking a toxin and working a model around it was a worthwhile possibility, especially if using a toxin like lead where there is a great deal known about it already. This idea was well received by all participants and Dr. Pruett noted that there might be enough epidemiological studies available for lead to try developing a model in that manner.

2.6 Considerations for Addressing Highly Susceptible Subgroups with Quantitative Microbial Risk Assessment

The presentation by Mr. Jeffrey Soller, Soller Environmental, addressed the use of quantitative microbial risk assessment (QMRA), specifically with regards to highly susceptible subgroups. He provided brief background information on the definition and components of QMRA, and on how the risks from microbes differ from the risks from chemicals. He defined microbial risk assessment as, "the process that evaluates the likelihood of adverse human health effects that can occur following exposure to pathogenic microorganisms or to a medium in which pathogens occur." He then provided background information on dose response, presented representative dose response data, and described models such as the Beta Poisson dose response model and the exponential dose response model.

Mr. Soller then discussed the prevailing conceptual models for risk characterization – individual level (static) and population level (dynamic). The individual level model estimates the probability of infection to an individual from a single exposure event and assumes that recurring exposures are independent. The population-level model estimates the number of

infections attributable to a specific exposure and does not require that exposure events are necessarily independent.

Differential susceptibility in QMRA can be addressed in both the individual and population level models. The scope of consideration depends on the perspective (individual or population) from which one considers the risk. From the both the individual and population perspective, the considerations include modulating the dose response relation, identifying the group-specific morbidity ratio, and identifying the group-specific measure of severity. When assessing population-based risk, the following additional issues are considered: duration of the infection/illness, background level of the infection, intensity of the pathogen passage, duration of incubation, and duration and effectiveness of immunity.

Mr. Soller provided examples of how QMRA can be used to address differential susceptibility. The examples included the following:

- An E. coli outbreak that occurred from school lunches in Japan that resulted in different dose response relations for students and teachers (Teunis et al., 2004).
- Incorporating immunity and infectious disease dynamics in dose response modeling of *Campylobacter jejuni* (McBride and French, 2006).
- Reported SARS outbreak in Hong Kong (Riley et al., 2003) which illustrates the potential importance of super-spread events in disease transmission modeling.
- A hypothetical scenario of a population exposed to *Cryptosporidium* through recreation in reclaimed water. This example illustrated how the duration of illness can impact the total level of illness in a community attributable to a specific exposure event.

Mr. Soller concluded his presentation with a discussion of potential research areas to address. He noted critical data gaps in microbiological risks to children, elderly, and immunocompromised individuals, but acknowledged the ethical and public health considerations in examining those groups. He suggested that the feasible methods that are available for filling these data gaps included: experimental studies using animals and interspecies extrapolation, or observational studies using prospective and/or retrospective epidemiology data. Other areas of research interest included: the modulation of dose response; attempting to narrow down the list of the most important pathogens for characterizing risks to sensitive subgroups; and considering further acceptance of health burden analysis to account for the duration and severity of an illness.

Questions/Answers and Discussion

A workshop participant asked Mr. Soller if he equated illness and infection. He responded that he did not equate them and further stated that in identifying risk, it is important to note if the endpoint is illness or infection. For most pathogens included in risk assessments, the endpoint is infection, but for some, it is illness.

A participant commented that he was interested in identifying subpopulations that may be responsible for spreading disease. He wanted to know what risk management practices could be taken. Mr. Soller responded that although quarantine is the main risk management solution, there are others. For example, *Cryptosporidium* in drinking water can be managed by providing treatment beneath sinks for at risk populations, rather than treating water in the entire system to levels that would be protective for such subgroups.

The question was asked if it was possible to assess secondary spread potential by a particular category of pathogens. Mr. Soller responded that the best way to account for secondary spread potential was to look at outbreak data. For a given pathogen, outbreak data could be used to determine which portion of the population is exposed due to person-to-person contact, and what portion is due to the primary source.

A participant then asked how life stages were taken into account in microbial risk assessment. Mr. Soller responded that, currently, very little data are available to allow risk assessors to account for life stages. However, it may be worth finding specific incidents where data exist for different age groups, and comparing the number in the population to the number infected.

One attendee suggested that a high priority research goal should be to address the pathogens that are known to cause the most illnesses. CDC has published rough estimates that can be used as a starting point. As the Agency has a specific statutory obligation to address risks to children, an emphasis may be placed on characterizing risks to children.

2.7 Review of Presentations and Discussions

To begin the second day of the workshop, Dr. Michael Broder, EPA RAF, provided a summary of the previous day's presentations. He also created a list of the overarching issues he gathered from the presentations as potential starting points for discussion:

1. There are numerous components to the immune system (T-cell response, B-cell response, etc.).
2. Human data indicate that immune suppression can result in: increased rate of infection, increased duration, increased severity, and lower median infectious dose.
3. Immune status changes with life stage. Early life stage and late life stage are more susceptible to infection.
4. The use of rodent models has inherent limitations. Specifically, he noted that there are differences in the rate of maturation of organs, different rates of maturation during gestation, differences in pharmacokinetic rates, animal data tend to be on various organs, and human data tend to be on peripheral factors (T-cells, B-cells, granulocytes, etc.).

5. Use of rodent models for deriving dose response is a point of debate. To this point, animal models have not been a model of choice.

Following Dr. Broder's presentation, the question was raised as to what could be done with the existing epidemiological data and data mining for microbial data. Not everyone was in agreement with this option based on the amount and quality of data available. Therefore, a suggestion was made to prioritize the microorganisms and focus on the ones that are making humans sick. An additional suggestion was made to use the existing NTP (animal) data set and assess how that might be used in modeling, as was discussed in Dr. Pruett's presentation of the parallelogram approach.

3.0 WORKSHOP DISCUSSIONS

The following three sections summarize the discussions from the first and second day of the workshop and the teleconference held on March 20. Flip charts produced during the workshop and meeting notes from the discussion session are presented in Appendix G.

3.1 Discussion – Day 1

Dr. Andrew Rooney, EPA ORD/NHEERL, facilitated the plenary discussion held following the presentations on the first day of the workshop. He set the following goals for the workshop discussion: (1) prioritize parameters and factors in assessing risk of susceptible groups to pathogens and toxicants, (2) identify tools or methods to quantify or describe increased risk, (3) recognize issues and data needs/gaps, and (4) discuss ways to reduce uncertainty in risk assessments. The discussion that followed focused on identifying the data needed to meet these goals, as well as the means to obtain such data. A summary of the discussion, based on three main topics, is presented below.

3.1.1 Availability and Types of Data

Several sources of readily available data were suggested by a number of participants. The Center for Disease Control (CDC) has data on infectious disease incidences. The National Health and Nutrition Examination Survey (NHANES) is one of the largest and longest running national source of measured health data. However, NHANES may not include microbial exposure data. Other sources of data noted were case studies, such as those conducted for lead, the National Children's Study, and epidemiological studies. Retrospective epidemiological studies may have limited usefulness. To determine susceptibility, you need to ask if the response to an infectious agent is greater or less than to a different population at a same level of exposure. It is rare to have that information in older data, such as is found in these types of studies.

Types of data discussed included both human and animal data. Baseline human data are available, such as caretaker data for older adults. In a number of human studies, response to a vaccine is the endpoint, and the pre-vaccine antibody levels have been recorded. However, none of the workshop participants knew if epidemiological studies could provide similar information.

While immunizations are not usually followed up with titers, veterinary data or databases may be useful, as it is common for dogs to be titred following vaccinations.

Human data may possibly be obtained from studies associated with homeland security (nonpoint source and outbreak data) and the military (immunization data). Vaccine companies have clinical data on vaccine efficacies. Although their data are not meant to represent the population as a whole, and data are not published, they may be accessible. As the first response to a vaccine is an antibody response, some care would be needed in interpreting the data. However, for some parameters, even data on neutralizing response are useful. The National Academy of Sciences concluded in a biomonitoring paper, that not all the resources needed are in one single place, and work would be needed to integrate data from various sources.

It was further noted that human studies are limited in usefulness because the sample sets are generally very small compared to animal studies. However, it was also stated that the original study for *Cryptosporidium* had 30-40 subjects spread across all dose groups, and that the data were still useful.

It was agreed that if there was good benchmark data on exposure for humans, animal studies could be used in conjunction with the data. A question was asked if it would be useful to deliberately infect communally-housed animals to see how infection spreads. It was decided that, if animal data are used, understanding the individual responses on individual animals would be a better preliminary step. However, animal models that use “clean” laboratory-raised animals may not be realistic. An animal study on aged rats living in junkyards indicated that these rats were strongly resistant to disease. It was suggested that pets and domestic animals might be a good place to start looking for the solution. Pet insurance exists, which suggests that actuarial studies are being performed for pets.

3.1.2 Prioritization of Parameters and Factors

In discussing the parameters and factors that need to be addressed on assessing the risks to susceptible populations, it was first noted that chemicals that are in the public’s eye get the most funding for research. For that reason, lead, for which a large dataset is available, could be a useful target for study. However, people working in immunotoxicology and microbial risk assessment use infectious disease as their endpoints. Information on the susceptibility factors are limited. Instead of looking at particular chemicals, it may be better to look at existing datasets such as the NHANES dataset or CDC’s data on infectious disease incidences. These data can be used to determine particular risk factors in which EPA would be interested.

Discussions then focused on the need to prioritize the type of microorganisms used in risk assessments. The immunotoxicological community has historically used microorganisms that are easy to use in practice; however, these are not the same organisms that pose threats with respect to microbial risk assessment. Animal immunotoxicological studies with these types of microbial agents, if available, would be useful. Two potentially useful organisms are *Cryptosporidium* and adenovirus. Heterotrophs may also be worth examining. One participant noted that organisms associated with illnesses and noroviruses may be more interesting. In

addition, the CDC has data on food-borne diseases that show which pathogens are propagating in the United States. The Office of Water's CCL may also be useful in prioritizing organisms.

It was reiterated that the goal of the workshop was to develop a matrix of information, not to establish No-Observable-Adverse-Effect-Levels (NOAELs). A useful matrix would include 10 important microorganisms and 3 susceptible subpopulations. The matrix would describe the susceptibility associated with different microorganisms, as well as the reaction of the general population versus susceptible groups. However, before starting on such an endeavor, it would be important to describe how the information from the matrix could be translated to humans. Any conclusions would need to be validated using epidemiological data. A *Cryptosporidium* study that contains data for Human Immunodeficiency Virus (HIV)-positive individuals could be useful.

It was then noted that, in the older adult population, it would be difficult to isolate which factors are actually associated with susceptibility, even if susceptibility could be measured. Experts in infectious disease may be useful to consult in dealing with this issue. Dr. Schaub stated that, in his presentations, some of the responses are multi-factorial. Much of the response at any given age is iterative and previous events that occurred in each person's lifetime will continue to have an effect. This would be difficult to account for when examining data.

3.1.3 Risk Assessment Approach

The final part of the discussion focused on the approach to be used in the microbial risk assessment that would also reduce uncertainty and address variability. In performing risk assessments, the Office of Groundwater and Drinking Water monetizes benefits and utilizes the Maximum Contaminant Level Goal (MCLG) of zero for microbes. It was noted that this approach will not be sustainable in the future. Any regulation or any sort of risk management approach may require a higher level of demonstration of cost efficacy and protective efficacy.

One participant stated that there are conditions under which immunity and transmission should be considered together. For example, if risk management causes increases in person-to-person transmission, but immunity increases as well, the two factors might cancel each other. It was then suggested that, instead of trying to approach the problem looking at defining effects associated with a particular age or particular preexisting conditions, it may be possible to approach the problem looking at relative differences between conditions.

Currently, the Office of Water is reviewing risk assessment protocol components that may alter the normal method of risk assessment, such as new approaches or factors. Any new models that the workshop develops may be helpful in this regard. Instead of looking at individual risks, it may be better to approach the problem so that the most susceptible 25% of the population is protected.

It was indicated that drinking water advisories issued by the CDC are for specific waterbodies, not tapwater, and are not strongly worded. It was suggested that either the CDC is not doing a good job of providing risk advisories to high risk people, or that risk managers and

communicators are not conveying strongly enough the risks. However, it was added that the most susceptible populations, such as people with AIDS or bone marrow transplant patients, are carefully monitored by their physicians, and that it is probably not CDC's responsibility to base their assessments on those types of sensitive populations.

The Clean Drinking Water Act requires the best possible risk assessments, under practical considerations. EPA, however, has no policy regarding what proportion of the population to be protected. Although mandates exist for EPA to protect sensitive human populations, there has been no differentiation between sensitive and hypersensitive.

Although mild- to moderate- immunosuppression occurs more commonly in the human population than more severe suppression like AIDS, workshop participants questioned how the severely immunosuppressed subpopulation could be identified. HIV is measurable, but other types of immunosuppression are not easy to measure. On the other hand, it is a very atypical phenomenon. Because of the difficulty of finding the severely immunosuppressed subpopulation, it might be better to expend energy on better defining the uncertainty.

It was also noted that risk/benefit analysis is often monetized keeping in mind the "healthy" portion of the population. For example, justification for a universal vaccine used this type of analysis: calculating the cost of vaccination, the cost of side effects, and the cost of parents having to stay home from work to take care of sick children. In this case, the analysis is done for the healthy children, and the unhealthy portion was safely ignored during risk assessment, because factors apart from the individual children were taken into account.

3.2 Discussion – Day 2

Dr. Rooney also facilitated the discussion held on the second day of the workshop. The discussion, which followed a brief summary of the presentations and discussions from Day 1, focused on the two main topics presented in the charge questions (Appendix D), microbial risk and immunotoxicity, as well as supplemental issues related to data and research needs.

3.2.1 Microbial Risk

3.2.1.1 *Immunological Components*

The discussion first focused on the immunological components that could be applied to microbial risk assessment. It was noted that there is a marked difference in older and younger animals when exposed to intracellular pathogens, which involves T-cell immunity. Older animals respond similarly to younger animals when they are exposed to extracellular pathogens, which involve antibody response. In older animals, increased numbers of neutrophils and macrophages offered protection. As such, in examining the issues of life stages, infection, and susceptibility, examining one pathogen will not be sufficient, given the varieties of response associated with different pathogens. There are similar examples to be found from human studies. For example, mortality in children caused by respiratory infections are predominantly bacterial. Mortality in older adults caused by respiratory infection, in contrast, is caused by factors such as

influenza. Although mortality rates of adults increase with age, bacterial causes of mortality predominate in the very young.

It was further stated that, in older adults, the ratio of memory to naïve T-cells is greatly changed. Memory cells tend to be more resistant to xenobiotics (e.g., radioresistant, resistant to costerone). The T-cells in the thymus cortex are mostly naïve. In the medulla of the thymus are memory T-cells, which are quite resistant. T-cell immunophenotyping has been used to predict susceptibility in extreme cases of immunosuppression, such as AIDS or primary immunodeficiency diseases. It is not clear whether it can be used as a predictor for disease in cases of mild-moderate immunosuppression.

3.2.1.2 Interspecies Extrapolation and Uncertainty Factors

It was suggested that if specific immune parameters can be found that are measurable in both animal and humans studies, it might help with extrapolation from animals to humans. Delayed Type Hypersensitivity (DTH), which can be considered a measure of cell function, might be useful for looking at cell-type bacterial infections. There are DTH data in the NTP, and DTH can be obtained in humans with patch tests. Measurements of Natural Killer (NK) cells would be difficult to use, as the NK measurements do not state what proportion of the NKs have been activated to do work. It was noted that what is measured in animal studies is different from what is measured in human studies. For example, spleen cells are routinely used in the mouse or rat, while peripheral blood leucocytes are used in humans.

Interspecies extrapolation is usually broken into pharmacokinetic and pharmacodynamic components. PBPK models can be used to look at pharmacokinetic component. For the pharmacodynamic portion, there remains uncertainty. The key seems to be trying to mesh the maturation process in the rodents to the maturation process in adults. In IRIS, uncertainty factors are utilized for this reason. In some cases, there is sufficient data for a physiologically based pharmacokinetic model. On the microbial side, however, there is a lack of experiments looking at pharmacokinetic issues. Given that there are these problems/challenges associated with animal to human extrapolation, the animal studies address many issues of interest for human health associated with life stages. The rodent has many of the same physiological processes as humans, so it could be a useful model for determining susceptibility.

3.2.1.3 Susceptibility

It was indicated that different types of infections predominate in different age groups and that, in addition to the age-related differences in the immune system, other factors are responsible such as the pH of the gut. The problem can be divided into three components: life stage, immune status, and exposure. It was suggested that the problem of exposure be separated from the other two components. One approach would be to take an organism and determine the average rate of infection in a population. Once that is determined, a range of doses can be considered. A sensitivity analysis could determine which of the three components are most important for a specific pathogen. Evaluation of the immune status would then determine the

susceptibility. Identifying predictive markers would be most difficult in the older population because of the heterogeneity of the immune response that occurs in this population.

3.2.1.4 Risk Assessment Approaches

Dr. Luster suggested that many of the discussions up to this point were addressing issues that could be too complicated to be practicable. The issues needed to be simplified in a way that would allow public-health protective levels to be established. When approaching the problem, it may be worth exploring it from the opposite direction as well: first determine how susceptibility changes quantitatively with life stages and then determine the safe levels associated with a population.

It was noted that shifts in the dose-response curve, as well as the intensity of the disease may be considered when determining effects (severity, duration of sickness, etc.). The data currently available for host resistance assays tend to show incidence. It is particularly important if Disability-Adjusted Life Years (DALY) or Quality-Adjusted Life Years (QALYs) are used for calculating benefits. In addition, the manifestation of the disease may be more important than the proportion of the population with the disease.

The applicability of animal studies compared to epidemiological studies to address age-related differences to susceptibility from microbial agents was discussed in detail. It was felt that epidemiological data would be most useful to address this question, but there were insufficient epidemiological data currently available because of issues dealing with obtaining accurate exposure data including quantity ingested and contributions by non-microbial materials. For example, lead in recreational waters may cause suppression of immune function. Therefore, the level of pathogens causing infection would be lower if lead is present. One participant, however, was more optimistic about using epidemiological data for life-stage susceptibility. Since it is important to consider how any information obtained will be used, epidemiological data are potentially powerful. It was agreed that animal studies face several challenges, particularly in terms of extrapolation to humans, but is a good starting point as both the type and dose of the infectious agent can be relatively easily controlled and monitored.

It was noted that, historically, recreational water criteria were based on fecal coliform levels, using gross correlations to disease. However, in the near future it may be possible to monitor and establish criteria for specific pathogens. Thus, there are two very different existing approaches for risk assessors: relatively gross indicator versus enumerating specific pathogens.

Another approach for risk assessment is to use qualitative information to identify a particular subpopulation to be considered. If older adults, for example, are the population of interest, then animal or epidemiological data for that population can be used to determine rates of infection. This rate can then be used to develop a quantitative model. For example, if the Office of Water were to set pathogen-specific risk criteria, they would derive safe levels of pathogens in swimming waters, or acceptable risk levels. If the population of interest is children 12 and under, experiments could determine quantitative differences for young animals vs. mature animals, and the results could be extrapolated to humans. This information could then be used

by risk assessors to derive criteria, such that a specific concentration translates to a specific level of risk.

It was further indicated that non-microbial environmental factors may exist. For example, lead in recreational waters may cause suppression of immune function. Therefore, the level of pathogens causing infection would be lower if lead is present.

3.2.1.5 Uncertainty Factors/Data Gaps

A 10x uncertainty factor is routinely added in chemical risk assessment to protect the young. It was indicated, however, that this factor doesn't address persistent effects. Another participant stated that the uncertainty factor of 10x was not selected randomly and is based on the consideration of many different issues.

Dr. Haas was asked if the same species and strains of pathogens are used in animal models. Dr. Haas responded that different species and strains of pathogens are associated with different levels of susceptibility. If data are not available to address this issue, an uncertainty factor may need to be applied. It was also noted that uncertainty factors are not currently used in microbial risk assessment and development of such a paradigm may be difficult.

It was further recommended by an attendee that studies conducted on immature rodents should be taken in context of species differences in immune system development when translating the results to young humans. Different immune components in rodents mature at different periods of gestation compared to humans. For example, at birth a mouse is equivalent to a 3rd trimester human in terms of immunological maturity, though individual aspects differ. Secretory immunoglobulin A (Secretory IgA) continues to mature in humans until about 15 years of age, but reaches mature levels in rodents around day 21-30 postnatal.

3.2.1.6 Virulence and Persistence

In microbial risk assessment, definitions for "severity" and "persistence" are not well defined. In dose-response studies, a distinction is made between levels of GI illness. GI illness, for example, is typically defined as 3 or more loose stools. For duration of illness (persistence), measurements of the length of time people shed pathogens in their stools have been used. It was noted that outbreak reports contain information in terms of durations of illness and infections. For "virulence," not much has been done in an environmental context. Relative measures are used for definition – certain low infectious agents are considered synonymous with each other in terms of virulence.

It was also stated that animal post-resistance data are limited, although one study was mentioned, in which *Streptococcus* was introduced to animals. Generally, pathogens are chosen based on the pathogen's influence on parts of the immune process. Instead of looking at virulence, severity and persistence tend to receive the focus in animal studies. Matrix studies that involve different doses do exist. It was suggested that these studies would best approach the question of severity to infection and susceptibility.

It was suggested that two factors to be examined for environmentally-borne microbes: the resistance factor to the environment, and the virulence factor to the host. The differences in life stages are considered. For example, in the older adult population, a GI infection is more likely following exposure. The older population not only has less immune capacity, but also a higher pH in their stomach, resulting in a greater chance of pathogens surviving.

A question was raised concerning the delayed onset of health effects. None of the participants were aware of any immunotoxicants that have a delayed onset. Lead studies have shown that neonatal exposure can lead to effects that are not seen until much later in life. However, it was argued that perhaps, in those studies, the monitoring intervals were not frequent enough. It was noted that reduced birthweight is usually associated with reduced immune function. Immunotoxicity data in chronic studies or in aged animals are limited. In one study, dexamethasone was administered to pregnant animals, resulting in immunosuppression in both dams and children of comparable duration. This suggests an endocrine effect, persisting because of some special physiological consequences. The dam being pregnant may have opened up a critical window.

Currently, scientists tend to examine single microbes at a time rather than mixed infections. Mixed exposures may influence virulence. However, the participants agreed that changes in virulence factors and mixed exposures cannot be addressed at this time. Risk assessments in recreational waters are probably the best examples of mixed exposures. Generally, fecal matter is used as the measure for recreational waters with the common endpoint of GI illness.

3.2.2 Immunotoxicity

3.2.2.1 *Testing Guidelines/Endpoints*

The discussions on immunotoxicity began with an evaluation of the current testing guidelines and the applicability of humoral immune function and innate function endpoints in microbial risk assessment. Are additional data or tests needed to predict risks from intracellular pathogens? It was first noted that part of the guidelines developed by the Office of Pesticide Programs relate to age differences, but specify adult rodents be used. In addition to B cells, the antibody response can assess T-helper cell (Th) immunity, since the response is T-cell dependent. No direct cell mediated immune test (e.g., DHR) is currently incorporated in the test guidelines

Use of NK as measure of risk was then discussed. It was noted that the role of T cells, B cells and antigen presenting cells in the antibody response has been standardized, while NK data show a lot of variability. The workshop participants agreed that the NK data may not be the best measure of risk.

A question was raised concerning the availability and usefulness of measuring phagocytic function of neutrophils or macrophages in the lungs. While such data would be

important for inhalation-specific routes of exposure, it may not be supported for systemic effects. It was then noted that, in systemic suppression, polymorphonuclear neutrophils (PMNs) play a significant role. Current assays available to measure changes in PMN function are not particularly sensitive or easy to conduct. However, PMN counts are routinely measured as part of toxicity testing.

A suggestion was made to review the NTP data sets to determine how DTH and antibody response track together. It was noted that looking upstream in the immune response for markers may lead to biomarkers that are easier to measure, but those markers do not always translate into a functional decrease in immune response.

The workshop participants concluded that the humoral immune function endpoints in the current testing guidelines, such as T-cell dependent antibody response, may not be adequate to predict risk of infection with intracellular pathogens. Assessing guidelines deficiencies and the need for modifications were discussed. The current guidelines target young adults, not children or older populations.

3.2.2.2 *Data Gaps/Risk Assessment Approaches*

The discussion then turned to the absence of data, and risk assessment approaches. One participant stated that with pesticides, the approach has been to look for any evidence of immunotoxicological concerns in adult animals. If any are found, they are addressed with an uncertainty factor. If the effect is happening at low doses, it becomes a concern for risk assessors. It was noted that developmental exposure involves both severity and duration, and may result in a permanent developmental effect. This effect can be considered in a risk assessment. In cancer assessment, risk assessors consider early life exposures that may cause cancer later on. Asthma is also similar. These situations may be analogous to immune function.

In closing this part of the discussion, one participant indicated that, if effects on the immune system can be identified, models are available to do the risk assessment. This approach would simplify the entire process.

3.2.3 Research Needs/Data Sources

The first area of research discussed was the use of immunotoxicology studies to improve microbial risk assessment for age-related differences in susceptibility and whether such information can also be used to improve the process of immunotoxicological risk assessment. The research would help determine if developmental exposure to microbial pathogens has an effect on the immune response. Representative organisms, such as adenoviruses, might be used to test the hypothesis using rodent at various life stages administered with and without known immunotoxicants followed by challenge of microbial agents of interest. Ideally, model compounds would be used – compounds whose effects are well understood. It was suggested that, in examining pathogens, three classes are to be considered: viruses, protozoa, and microbes. Such information could be used to help establish margins of exposure (MOEs).

A participant indicated that the Office of Water is currently preparing a White Paper on sensitive subpopulations and pathogens. Input from the participants of this workshop would be greatly appreciated.

Another research need arises due to the fact that in many human immunotoxicology studies that have been conducted, dose-related changes in immune responses were measured that were statistically different from the normal population, but the values were still within reported normal ranges. Thus, the biological significance of the data were questioned. A large population study is needed, looking at disease incidences in the general population simultaneously with immune tests, to determine the quantitative relationship between small changes in the immune system and disease.

Possible helpful datasets that may help to address this issue are available from the National Institute of Allergy and Infectious Diseases (NIAID) Multicenter AIDS Study and NHANES. The former consists of 40,000 non-HIV infected individuals for whom immune tests and infectious disease records are available

Another important source of data noted was the National Children's Study (NCS). The NCS is a prospective study designed to examine the effects of environmental influences on the health and development of more than 100,000 children across the United States, following them from before birth up to adulthood. It is hoped that the study will yield information concerning prenatal infection. Environmental factors, stress, and microbial factors are all being considered in the study. It was suggested that immunological markers, such as white blood cell counts, are also needed, as well as vaccine responses. This type of information may be included from additional studies.

It was further noted that, in the absence of specific test data, one may be able to relate changes in antibody responses and increases in infection in animals for a given toxin via modeling. The animal data can then be used to detail the quantitative link between the change in antibody response and the change in human response.

In summary, it was suggested by Dr. Pruett that the microbial risk assessors in the workgroup create a list of pathogens that would be most relevant to the group, and perhaps design a research program to fill in data gaps between incidence and severity of infection. Since there are a series of epidemiological studies looking at recreational water, there may be a way to check for immunological status of the populations based on age groups.

To account for the older adult population, participants suggested examining data for veterans, medicare, and NHANES. It was also noted that Jack Colford at the University of California at Berkeley has intervention studies for large groups of adults over the age of 55. Other potential sources of data include State health departments and European governments. European governments may have national databases for safety data standards. However, it was indicated that if such data exist, it would be in NHANES or one of the NCHS databases.

3.3 Teleconference Discussion

A follow-up conference call was held on March 20, 2007, among approximately 15 members of the workgroup (primarily from EPA) to discuss the major recommendations from the workshop and to determine future directions. Mr. Gary Bangs served as facilitator for the call. Workgroup members considered a variety of activities to further advance the state-of-the-science toward integration of immunotoxicity, microbial, and life stage information for risk assessment. Potential future directions include: designing and conducting a research study; developing a case study; examining possible assessment approaches including using QALYs/DALYs; characterizing uncertainty; and conducting sessions at major scientific conferences to obtain additional input from the scientific community.