

Integrating Life Stage Susceptibility into Immunotoxicity and Microbial Risk Assessment

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Regulatory Interest in Life Stage and Susceptibility

- “Pesticides in the diets of infants and children” (NRC, 1993)
 - Need for data on the effects of pesticides on the developing reproductive, immune, and central nervous systems.
- Food Quality Protection Act (EPA, 1996)
 - Separate assessment for infants and children to establish pesticide residue levels
- Safe Drinking Water Act (EPA, 1996)
 - EPA to conduct studies to identify sensitive subgroups
- Executive Order # 13045, 1997
 - Identification of potential health risks to kids a high priority

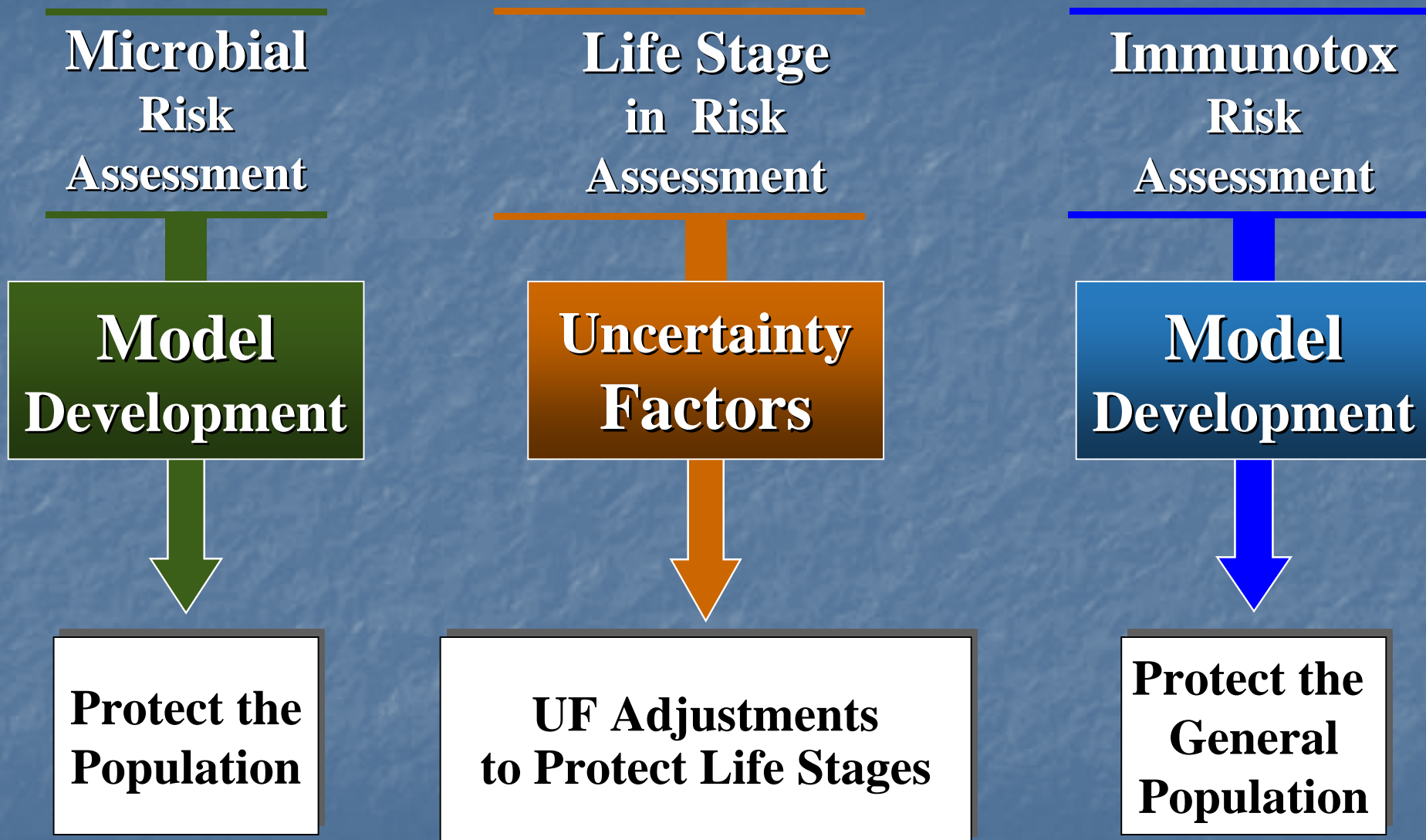
Agency Interest in Life Stage and Susceptibility

- ORD Human Health Multiyear Research Plan, Long-Term Goal 3: Susceptible Subpopulations -
 - Is there differential life-stage responsiveness or exposure to environmental agents
 - What are the long-term effects of developmental exposure to chemicals?
 - How does aging affect responsiveness to environmental chemicals?

Agency Interest in Life Stage and Susceptibility

- **ILSI/HESI Technical Panel: Agricultural Chemical Safety Assessment**
 - Enhanced F-1 component of the traditional two generation reproduction study
 - Includes developmental immunotoxicity and developmental neurotoxicity endpoints
- **Microbial Contaminant Candidate List Workgroup**
 - Takes life stage and susceptible groups into account in screening criteria

Current Practice

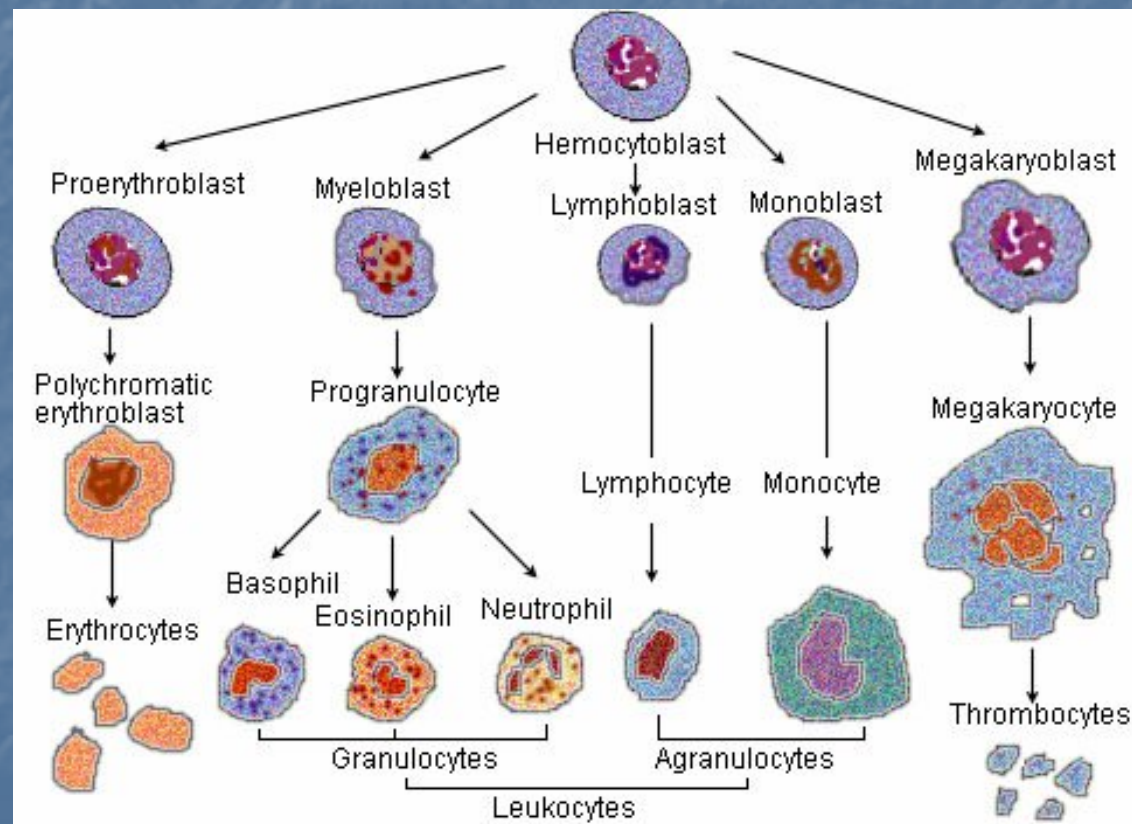


Basics of Immunology

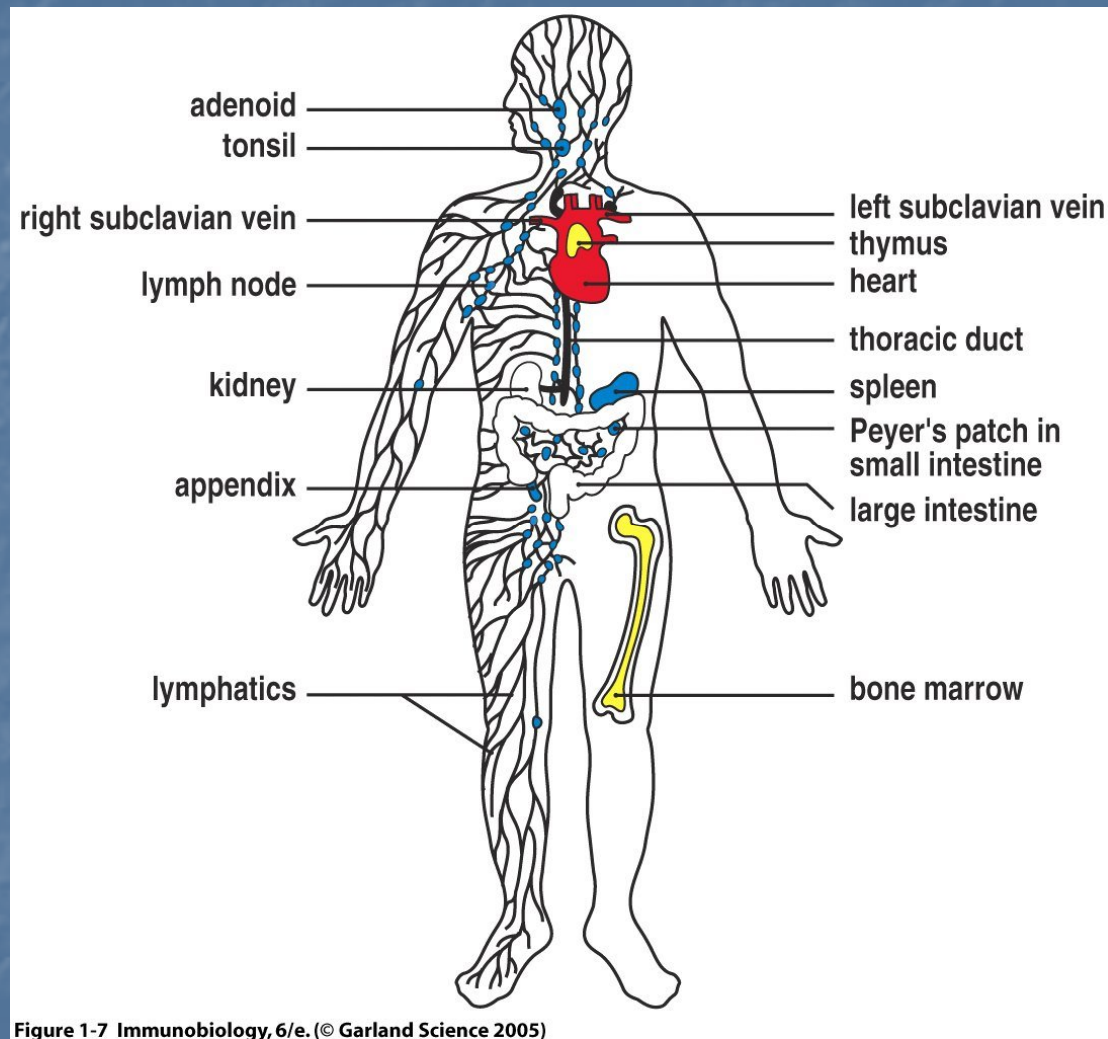
Bob Luebke
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Integrating Immunotoxicological and Microbial Risk Assessment for Susceptible Life Stages

Bone Marrow: Source of Immune System Cells



Immune System Anatomy



Basics of Immunology

The Immune Response



Innate Immunity

- Phylogenetically ancient
- Rapid (minutes – hours)
- No cell proliferation required
- Limited recognition
- Limited memory (? mammals)

Adaptive (Acquired) Immunity

- First appeared in jawed fishes
- Slow (days)
- Requires proliferation and differentiation
- Infinite array of specificities
- Long-lasting memory

Basics of Immunology

- **The adaptive immune response to antigen**
 - Recognition as foreign
 - Antigen processing and presentation
 - Gene transcription, mediator release, cellular proliferation and differentiation, effector protein synthesis
 - All steps must work properly or function will suffer

Basics of Immunology

- **Cells of the adaptive immune system**
 - **B lymphocytes: differentiate into plasma cells, produce antibody**
 - **T lymphocytes: mediator production, cytotoxicity, regulation**
 - CD4+ T helper cells, T regulatory cells
 - CD8+ Cytotoxic T cells, T suppressor cells

Host Factors Influencing Resistance to Infection

- Age
- Gender
- Genotype
- Nutritional status
- Life style choices
- Life events
 - Acute toxicity
 - Stress response in lab animals

Life Stage and Immunocompetence

- The developing immune system
 - Innate immunity
 - Neutrophils
 - Provide 1st line of defense
 - Phagocytosis and killing, particularly extracellular bacteria
 - Lower rate of production in the bone marrow (easily depleted)
 - One third to ½ the content of bactericidal proteins vs adults
 - Complement system
 - Lysis of Ab-coated cells
 - Opsonization of encapsulated bacteria
 - Neonates have 60-80% of adult levels
 - NK cells
 - Nonspecific killing of certain tumor cells and some infectious agents
 - One third the number as adults (cord blood)
 - Bind only 2/3 as many targets as adults
 - Of those that bind, killing is about half adult level

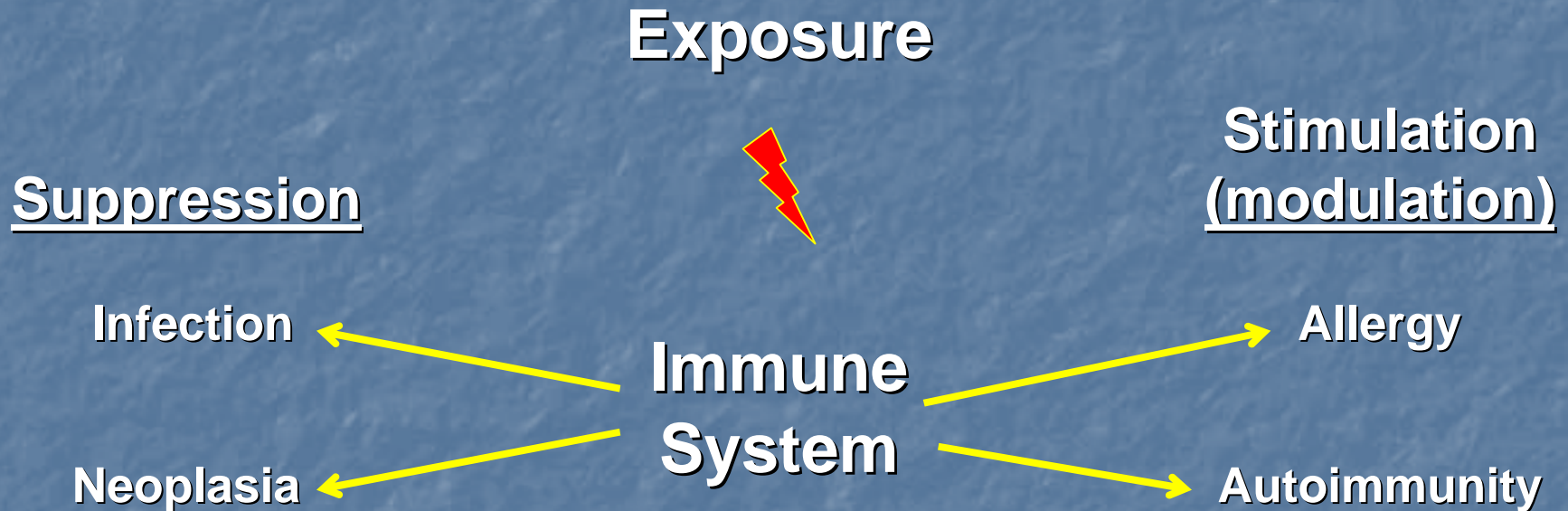
Life Stage and Immunocompetence

- The developing immune system
 - Adaptive immunity
 - Humoral immunity
 - High level of protection via maternal IgG that wanes with age (by ~60% at 3 months)
 - IgM and IgG levels reach 50% of adult levels by 7-12 months; IgA by 3-5 years
 - Cellular immunity
 - 90% of neonate T cells are naïve
 - “Allergic” phenotype of cytokine production is the default at birth; decreased resistance to intracellular bacteria
 - Resistance to infection
 - Reduced innate and adaptive function increases susceptibility to:
 - Encapsulated organisms: e.g., group B *Streptococcus*, *Haemophilus*
 - Intracellular organisms: e.g., *Listeria*
 - Viral infections, e.g., influenza

Life Stage and Immunocompetence

- **The aged immune system**
 - **Innate Immunity**
 - **Neutrophils**
 - Normal numbers
 - Increased rate of apoptosis
 - Ingest fewer bacteria/cell; less adept at killing ingested organisms
 - **Adaptive immunity**
 - **Humoral immunity**
 - Fewer Ab-producing cells
 - Less Ab produced/cell
 - Lower quality Abs are produced
 - **Cellular immunity**
 - Generalized decrement in function on a per cell basis
 - Decreased ratio of naïve to memory T cells
 - **Resistance to infection**
 - **Reduced innate and adaptive function increases susceptibility to:**
 - Encapsulated organisms, e.g., group B *Streptococcus*, *Haemophilus*
 - Intracellular organisms, e.g., *Listeria*
 - Viral infections, e.g., influenza

Xenobiotic Exposure and Immunocompetence



Consequences of Xenobiotic Exposure on Immunocompetence

- “Chemical AIDS”...not
 - Severe immunosuppression associated with opportunistic infections
 - Uncommon at the population level
 - HIV/AIDS
 - Severe primary immunodeficiency
 - Bone marrow and organ transplant patients
 - T helper (CD4⁺) counts decreased to < 500/μl (normal=1500-2500)
- Most likely outcome: mild to moderate immunosuppression
 - Recovery expected when exposure ends
 - Immune system redundancy
 - Immune system self-renewing
 - Developmental effects may be an exception

Immune Mediated Resistance to Infection

- Extracellular organisms (antibody, phagocytic cells)
 - *Staphylococcus, Streptococcus, Escherichia*
 - Certain helminths and protozoa
- Intracellular organisms (phagocytic cells, T cells)
 - *Listeria, Mycobacterium*
 - Viruses
 - Certain helminths and protozoa
- Toxic products or constituents (antibodies)
 - Tetanus toxin
 - Diphtheria toxin
- Transformed (neoplastic) cells (cytotoxic T cells, NK cells)

Organism Factors Influencing Host Resistance

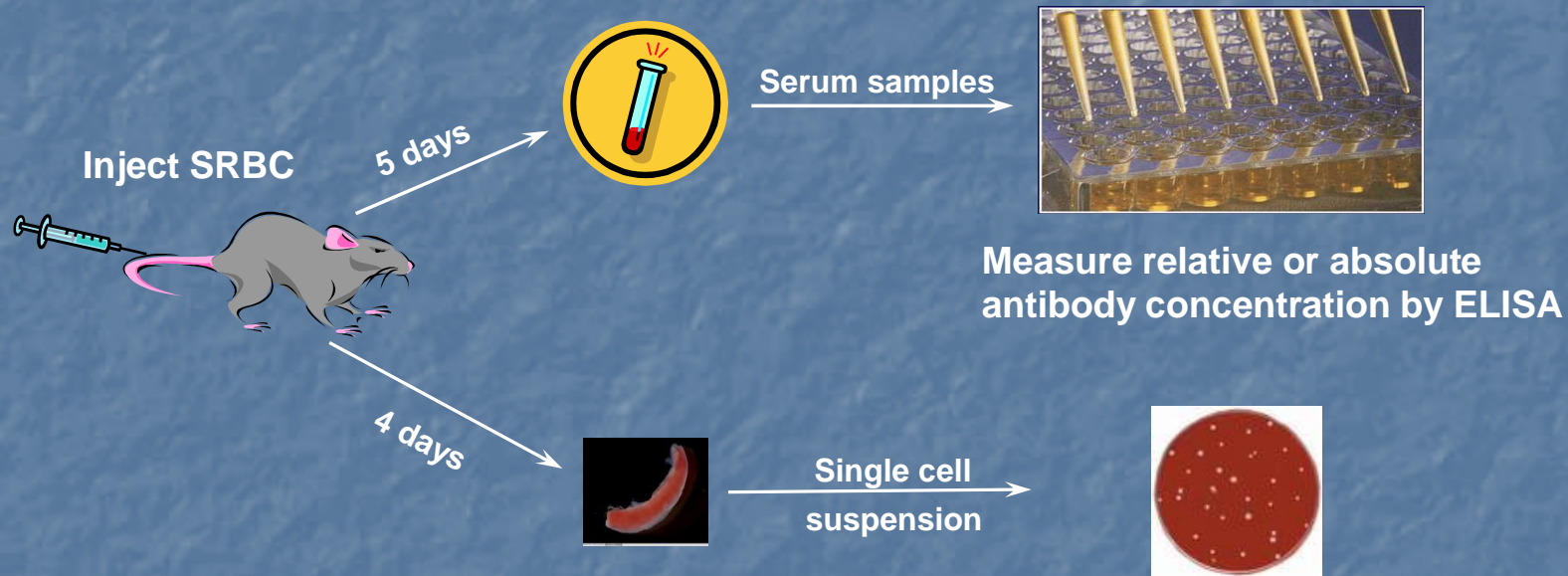
- Dose of the organism
 - Few: rapidly eliminated
 - Many: overwhelms innate and adaptive resistance
 - May cause colonization
 - May cause disease
- Virulence factors
 - Ability to evade detection or destruction
 - Production of toxins and adherence factors
 - Rapid growth
 - Very low infectious dose
 - Noroviruses
 - *Cryptosporidium* (certain strains)
 - *Giardia*

Immunotoxicology Hazard ID

- Research in government and academic labs
- Nomination for testing by NTP
- Observations in routine toxicity testing
 - Mass, cellularity, architecture of spleen and thymus
 - Abnormal hematology

Immunotoxicity Hazard ID

EPA Health Effects Test Guidelines: OPPTS 870.7800
Immunotoxicity (TSCA, FIFRA)



Optional Assays

Phenotypic analysis
(if TDAR negative)
NK cell activity

Immunotoxicity Risk Assessment

- Draft immunotoxicity risk assessment guidelines
 - Suppression
 - Functional deficits considered more significant than observations
 - Dose response
 - Recognition of age-related sensitivity/susceptibility

Life Stage Sensitivity to Immunotoxicants and Risk Assessment

- Developmental vs. adult exposure to xenobiotics (DES, DZP, Pb, TCDD, TBTO)
 - Can cause long-lasting (\pm lifetime) effects at doses that cause short-term immunotoxicity in adults (e.g., DES, \pm DZP)
 - Testing adults will detect effects, but grossly underestimate the relative risk of gestational/neonatal exposure
 - Can cause immunotoxicity at lower doses in the young than in mature animals, and effects are somewhat persistent (DZP, Pb, TBTO)
 - Application of an uncertainty factor may provide protection, but would not predict persistence
 - Can cause long-lasting (\pm lifetime) effects in offspring at doses that do not affect immune function in adults (e.g., TCDD in rats)
 - Testing adults only would fail to detect immunotoxicity

Implications for Risk Assessment in Sensitive Groups

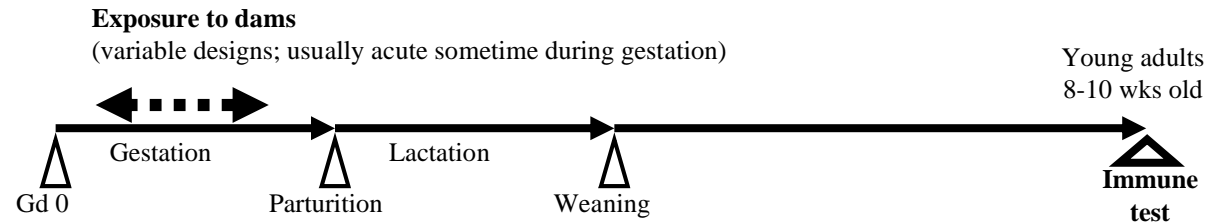
- Persistence of effects
 - Simple delayed maturation
 - Incomplete maturation with long term consequences
- Critical windows of developmental sensitivity
 - Immune system maturation in rodents and humans

Comparative Ontogeny

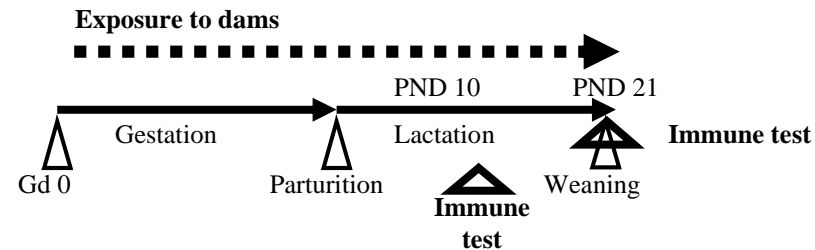
Event	Mouse (days) (% of term)	Human (weeks) (% of term)
Fetal liver begins functioning as a hematopoiesis site	10.5 (50%)	6 (15%)
Appearance of T cells in fetal liver	14 (67%)	6-8 (15-20%)
Organogenesis of thymus begins	11 (52%)	6 (15%)
Secondary lymphoid organs begin to develop	10.5 (50%)	7 (18%)
Lymph nodes start to appear	10.5 (50%)	8-12 (20-30%)
Spleen develops	13 (62%)	10-14 (25-35%)
B cell lymphopoiesis begins in bone marrow	17 (81%)	12 (30%)
B lymphocytes detectable in blood	13 (62%)	12 (30%)
CD4+ and CD8+ T cells detectable in spleen	19 (91%)	14 (35%)
Thymus development completed	13 (62%)	15-16 (37-40%)
Bone marrow becomes the major site of hematopoiesis	17.5 (83%)	22 (55%)
T cell receptor expression in periphery	Early post-natal	23 (58%)

DIT and Hazard ID

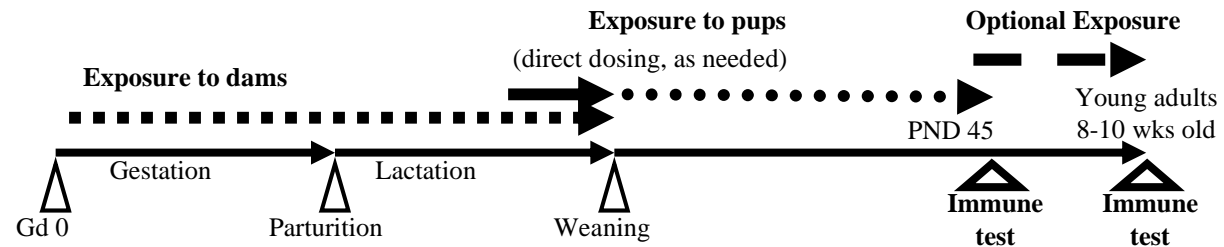
A. "Conventional" DIT Protocol



B. "Alternative" DIT Protocol



C. Proposed DIT Protocol for "all" critical windows

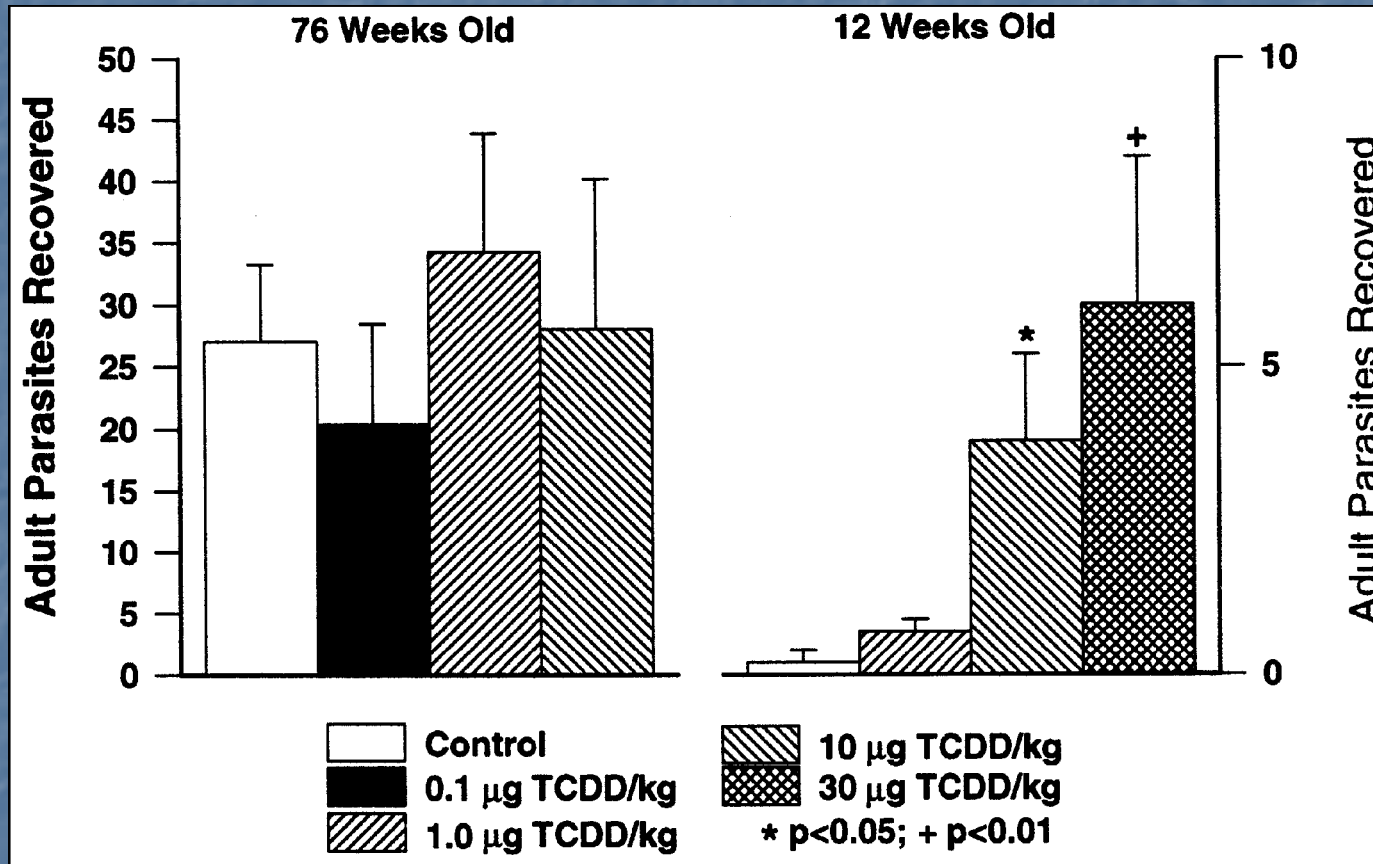


from M.P. Holsapple et al.

Implications for Risk Assessment

- In the young
 - Developmental exposure may result in persistent immunosuppression
 - Late gestational exposure in rodents mimics early to mid-gestation in humans
- In the aged
 - Stress affects resistance to infection
 - Chemicals?
 - Critical data gap

Advanced Age, Chemical Exposure and Host Resistance



Luebke et al. 1999. Toxicology 136, 15-26.

Mice

Altered Host Resistance in Humans

- **First Nations (Inuit) in Canada**
 - High level maternal exposure to chlorinated compounds in diet
 - Greater rate of inner ear infections in children
- **Dutch school children**
 - Grouped by PCB concentration in breast milk
 - Increased rates of inner ear infections at highest exposure levels
- **Elderly populations**
- **Stressed populations**

Issues to Consider

- “Normal” individuals are susceptible to infection
 - Host factors
 - Properties and dose of the infectious agent
- Susceptibility to infection in individuals exposed to xenobiotics
 - Host factors
 - Properties and dose of the xenobiotic and of the infectious agent
 - Detecting increased susceptibility at the population level is difficult
- Protecting the public health
 - Who do we protect?
 - If we protect the most susceptible, will we protect the entire population?
 - Can life stage immunotoxicity data augment microbial risk assessment?

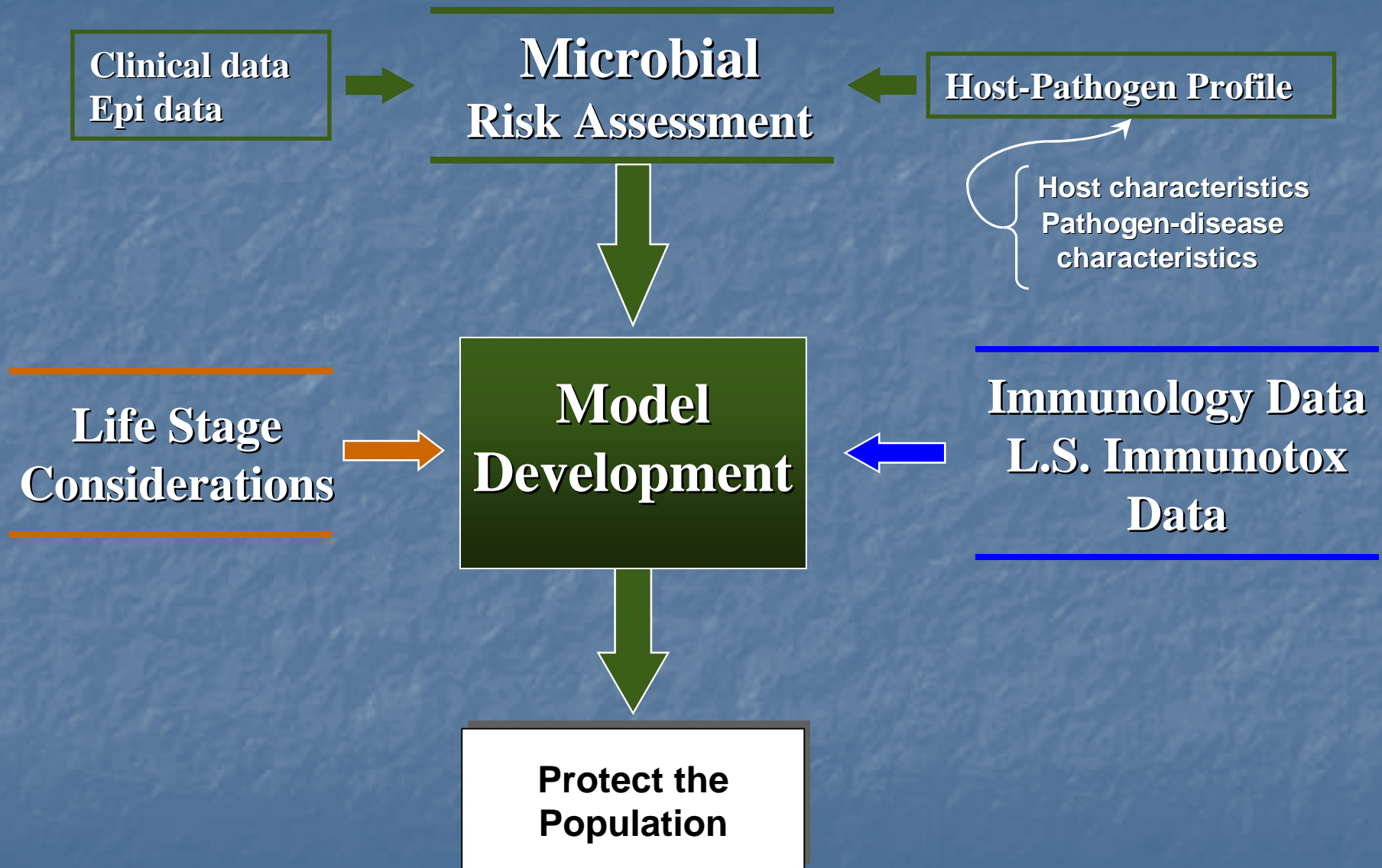
Challenges to Incorporating Life Stage

- **Into microbial risk assessment**
 - Are the models sensitive to small changes at the population level?
 - Outbreaks captured well, but are changes in incidence or severity of common infections?
 - Are default assumption adequate predictors of life stage sensitivity?
 - Can current models incorporate developmental immunotoxicity data?

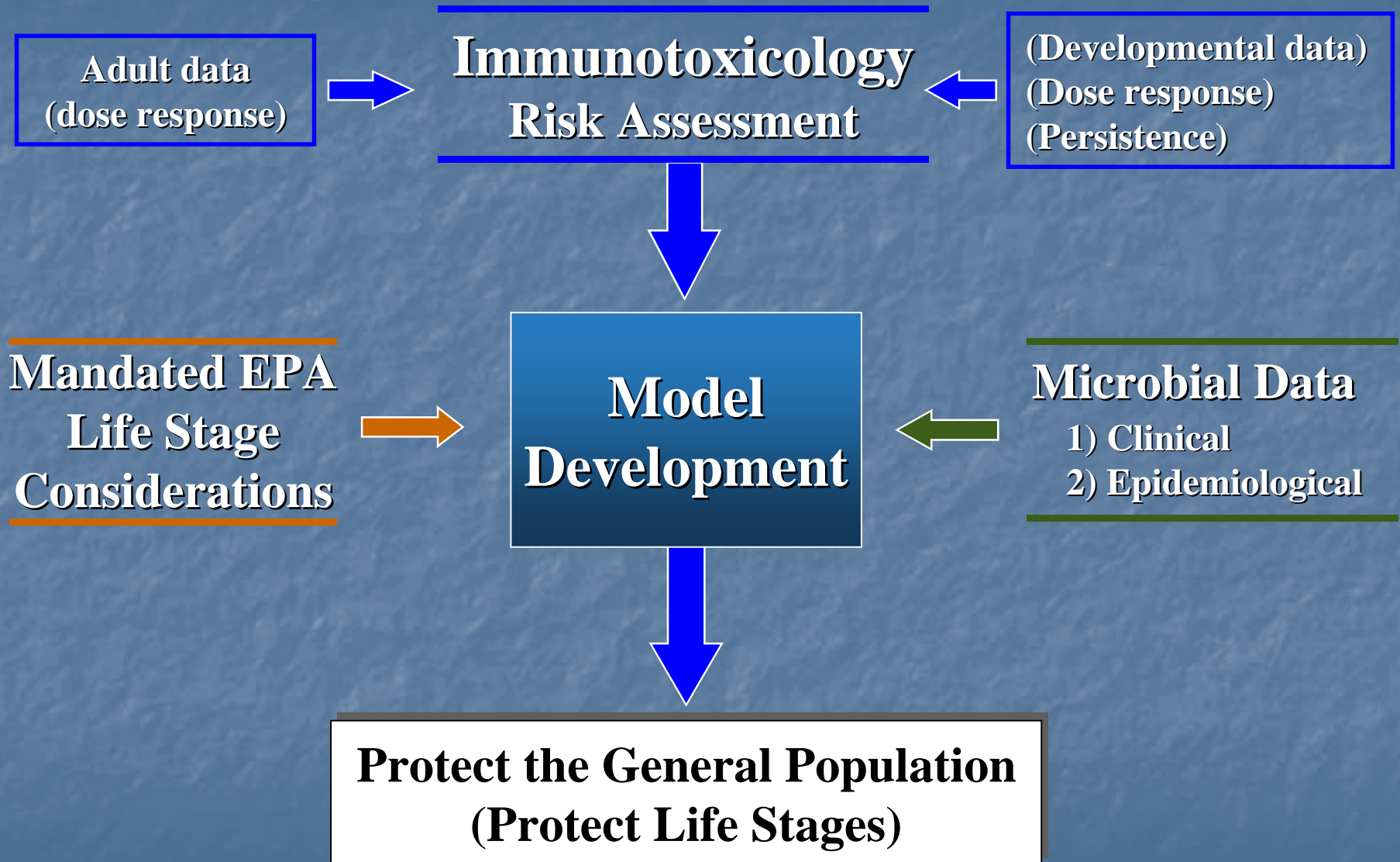
Challenges to Incorporating Life Stage

- **Into immunotoxicity risk assessment**
 - **Extrapolation of infection severity data in animals vs. incidence AND severity data in humans**
 - Animal data: young adults under ideal conditions
 - Human data: various age groups under uncontrolled conditions
 - **Extrapolation of lymphocyte data from animals and humans**
 - Animal data primarily from lymphoid organs (spleen and thymus)
 - Human data primarily from peripheral blood
 - Differential distribution of cell types

Emphasis on Microbial Risk Assessment



Emphasis on Immunotox RA



Potential for Improved Risk Assessment

**Microbial
Risk Assessment
Data**

**Immunotoxicology
Risk Assessment
Data**

**Scaling of
Uncertainty
Factors?**

**Protect General Population and
Susceptible Life Stages and Pops.**