NTP
National Toxicology Program

Levels of Evidence Criteria for NTP Immunotoxicology Studies

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Purpose of the NTP Immunotoxicology Testing efforts

- Develop and validate methods to evaluate modulation of immune function
- Evaluate immunomodulatory potential of agents of concern using a tiered testing panel
- Define cellular and molecular events associated with modulation of immune function

Impact of the NTP Immunotoxicology Testing efforts

- Tiered testing panel which has been the basis for regulatory guidelines and guidance in the field
- Leadership in evaluating predictive methods
- Risk assessment and extrapolation to human health

Source of Nominations

- NTP Chemicals of Interest
- Other State and Federal Agencies
- Interagency Committee for Chemical Evaluation and Coordination (ICCEC)
- Academic Community
- General Public

Range-Finding Studies to Screen for Immunomodulatory Effects

- 28-day studies
- Immunopathology
- Clinical Pathology
  - Complete Blood Count, Hematology
- Cell Mediated Immunity
  - Cytotoxic T Lymphocyte Assay
- Humoral Mediated Immunity
  - Antibody Forming Cell Assay
- Non-Specific Immunity
  - Natural Killer Cell Assay
- Cell Quantification
  - B and T lymphocytes (total and subpopulations), Natural Killer cells, Macrophages

Tissues that are collected for histopathology

- Thymus
- Spleen
- Lymph Nodes (mesenteric and popliteal)
- Bone Marrow (femur)
- Liver
- Kidney with adrenals
- Gastrointestinal tract with Peyer's patches

Standard Design for Range-Finding Study

# of Animals	Treatment	Designation
8	Vehicle	VH
8	Test Article	D1
8	Test Article	D2
8	Test Article	D3
8	Test Article	D4
8	Test Article	D5
8	Cyclophosphamide (Positive Control)	PC1
8	Additional Positive Control	PC2

Full Protocol Studies to Evaluate Immunomodulatory Effects

- Immunopathology, humoral mediated immunity, and cell quantification from range-finding studies plus additional tests to assess:
  - Cell mediated immunity
    - MLR response
  - Non-specific immunity
    - Macrophage function assays
  - Humoral mediated immunity
    - Antigen specific IgG
  - Host resistance
    - Dependent on the identified target in the range-finding studies

Levels of Evidence Criteria - Background

- The NTP has long employed specific conclusion statements, that are approved by the NTP BSC, for its "Toxicology and Carcinogenesis" studies.
- These conclusion statements represent a "level of evidence" sentence with regard to carcinogenic potential for each sex within each individual study.
  - Clear evidence
  - Some evidence
  - Equivocal evidence
  - No evidence
  - Inadequate study
- Such an approach allows for comparisons of different studies on the same test substance and for comparisons of conclusions across studies, to ensure similar criteria are employed uniformly.
- The NTP has developed guidance notes as to how these criteria should be applied.

Some Issues NTP Considered in Developing Draft Criteria

- Conclusions statements for NTP studies are hazard-based, not risk-based, to facilitate comparison across test substances for the same study types.
- Many of NTP's non-cancer toxicity studies include multiple (inter-related) endpoints - different from cancer studies.
- Applying the NTP cancer study "levels of evidence" approach to non-cancer studies would require some "finessing" to achieve the desired level of consistency.
- NTP staff recognized the desirability to use a graded (hazard identification) "level of evidence" scheme for expressing conclusions.
  - Any "positive" response should not result in the highest level.
  - Weight of study evidence approach.

Some Issues NTP Considered - 2

- NTP considered those endpoints that affect overall system function to merit the highest level of evidence ("clear evidence" of toxicity).
- Examples of such functional outcomes would be:
  - A positive result in a host resistance assay (not just a change in specific lymphocyte counts) for immunotoxicity.
  - A decrease in litter size (and not just a decrease in sperm count) for reproductive toxicity.
- Clear positive or negative results should be straightforward in applying the criteria. Findings at the boundaries would present more difficulty.

Immunotoxicology Criteria Working Group Meeting

- Held August 13-14, 2008, in Crystal City, Virginia.
- Working Group was presented background materials, a draft of the levels of evidence criteria, and case studies designed to test the utility of the draft criteria.
- Charge to the Working Group:
  - Evaluate the suitability and utility of the proposed criteria for describing the results from individual NTP immunotoxicology studies to indicate the strength of the evidence for their conclusions.
- Participants reviewed the case studies on their own and then met for group discussions.
- The draft criteria were revised by the Working Group based on their application in the case studies and the deliberations that followed.

Composition of BSC Working Group

- Comprised of stakeholders from the NTP BSC, Academia, Industry, and Government.
- Practitioners
  - Experts familiar with the nuances of study types, conduct, and data interpretation.
- Users of NTP study data
  - Representatives from regulatory bodies with experience in reviewing data from the specific study types.
- Some NTP staff were present at the working group meetings as technical advisors, but did not participate in the review.

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Immunotoxicology Criteria Working Group Participants

- Dr. Nancy Kerkvliet, Oregon State University, Chair
- Dr. Robert Benson, US-EPA
- Dr. Scott Burchiel, University of New Mexico
- Dr. Jeanine Bussiere, Amgen, Inc.
- Dr. Vicki Dellarco, US-EPA
- Dr. Rodney Dietert, Cornell University
- Dr. Michael Holsapple, ILSI Health and Environmental Sciences Institute
- Dr. Robert House, DynPort Vaccine Company LLC
- Dr. L. Peyton Myers, US-FDA
- Dr. Mitzi Nagarkatti, University of South Carolina
- Dr. Peter Thomas, Covance Inc.
- Dr. Michael Woolhiser, Dow Chemical Company
- Dr. Yung Yang, US-EPA
- Advisors to the Working Group
  - Dr. Michael Luster, NIOSH
  - Dr. Kimber White, Virginia Commonwealth University

Post Working Group Activities

- Working group reports approved by the NTP Board of Scientific Counselors (BSC) with comments (November 2008).
- Draft criteria reviewed and approved by the NTP Executive Committee (EC) (December 2008).
- Draft criteria reviewed and revised by NTP staff.
  - Addressing BSC and EC comments.
  - Consistency among disciplines and harmonization of language.

Levels of Evidence for Evaluating Immune System Toxicity

- Clear Evidence of Toxicity to the Immune System
  - Is demonstrated by data that indicate a dose-related[1] effect (considering the magnitude of the effect and the dose-response) on more than one functional parameter and/or a disease resistance assay that is not a secondary effect of overt systemic toxicity, or
  - Is demonstrated by data that indicate dose-related effects on one functional assay and additional endpoints that indicate biological plausibility

[1] The term "dose-related" describes any dose relationship, recognizing that the test article-related responses for some endpoints may be non-monotonic due to saturation of exposure or effect, overlapping dose-response behaviors, changes in immunologic manifestation at difference dose levels or other phenomena.

Levels of Evidence for Evaluating Immune System Toxicity:

- Some Evidence of Toxicity to the Immune System
  - Is demonstrated by data that indicate a dose-related effect on one functional parameter with no other supporting data, or
  - Is demonstrated by data that indicate dose-related effects on multiple observational parameters without robust effects on a functional immune parameter or a disease resistance assay, or
  - Is demonstrated by data that indicate effects on functional parameters or a disease resistance assay that are not dose-related, with other data providing biological plausibility.

Levels of Evidence for Evaluating Immune System Toxicity:

- Equivocal Evidence of Toxicity to the Immune System
  - Is demonstrated by data that indicate effects on functional parameters or a disease resistance assay that are not dose-related, without other data providing biological plausibility, or
  - Is demonstrated by data that indicate dose-related effects on a single observational parameter without effects on a functional immune parameter or a disease resistance assay, or
  - Is demonstrated by data that indicate effects on the immune system at dose(s) that produce evidence of overt systemic toxicity, or
  - Is demonstrated by data that are conflicting in repeat studies.

Levels of Evidence for Evaluating Immune System Toxicity:

- No Evidence of Toxicity to the Immune System
  - Is demonstrated by data from studies with appropriate experimental design and conduct that are interpreted as showing no evidence of biologically relevant effects on the immune system that are related to the test article.
- Inadequate Study of Immune System Toxicity
  - Is demonstrated by a study that, because of major design or performance flaws, cannot be used to determine the occurrence of immune system toxicity.

Key points to consider in applying the levels of evidence criteria

- Immunotoxicity is defined in the context that immune responses can be enhanced or suppressed by toxicants. As such, dose-related effects consistent with immunosuppression and immunostimulation will be considered in hazard identification.
- Functional effects, as defined as an alteration in the ability of the immune system to respond to a challenge or stimulus, should usually be weighed more heavily than observational parameters such as alterations in cell counts.
- Increases in severity and/or prevalence (more individuals with the effect) as a function of dose generally strengthen the level of evidence, keeping in mind that the specific manifestation may be different with increasing dose. For example, histological changes at a lower dose level may reflect deficits in immune function at higher dose levels.
- Biological plausibility for immunotoxicity must be considered in the context of the nature of the response, the magnitude of the response, and the pattern of the response, as well as the current understanding of immune system structure and function.

Key points to consider with the levels of evidence criteria - continued

- Insights from supportive studies (e.g., toxicokinetics, ADME, computational models, structure-activity relationships) and immunologic findings from other *in vivo* animal studies (NTP or otherwise) should be drawn upon when interpreting the biological plausibility of a change.
- The characterization of immunotoxicity must consider the impact of overt toxicity (e.g., effects on the immune system are not the direct effects of test article treatment, but are indirect effects mediated via stress and/or other dose-related responses).
- The characterization of immunotoxicity must consider the intended pharmacology of the test article. Immunotoxicity is reserved for unintended immunosuppression or immunostimulation.
- Results in one species or one sex are considered sufficient for evidence of immunotoxicity.

Implications for Adoption of the New Criteria

- More consistency in the conclusions from NTP studies of reproductive, developmental and immunotoxicity.
- There have not been previous attempts to develop such criteria for these study types.
- Potential for the studies to be noted as "authoritative" by certain regulatory bodies (eg Prop 65 – California OEHHA) like the cancer studies.
- Requisite expertise on the NTP BSC (or BSC sub-committees) for review of studies.
- Potential adoption by other groups.

An Example of a Conclusion Statement using the Immuntoxicity Criteria

- Under the conditions of these 28-day oral gavage studies, there was *clear evidence of toxicity to the immune system* in female B6C3F1 mice following exposure to *Substance X* based on the dose-dependent decrease in the antibody response to the T-dependent antigen in sheep red blood cells, the dose-dependent decrease in the cytotoxic T lymphocyte response to tumor cell challenge, and the decreased resistance to bacterial infection with *Streptococcus pneumoniae.*