NTP
National Toxicology Program

Levels of Evidence Criteria for NTP Immunotoxicology Studies

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Immunotoxicology Criteria Working Group Meeting

- August 13 - 14, 2008
- Crystal City Marriott
- Arlington VA

Immunotoxicology Criteria Working Group (ICWG)

- Nancy Kerkvliet *(Chair, BSC member), Oregon State University*
- Mitzi Nagarkatti *(rapporteur), University of South Carolina*
- Michael Woolhiser *(rapporteur), Dow Chemical Company*
- Robert Benson, *ODW, US EPA*
- Scott Burchiel, *University of New Mexico*
- Jeanine Bussiere, *Amgen*
- Vicki Dellarco, *OPP, US EPA*
- Rodney Dietert, *Cornell University*
- Michael Holsapple, *ILSI, HESI*
- Robert House, *Dynport Vaccine Company*
- L. Peyton Myers, *CDER, FDA*
- Peter Thomas, *Covance, Inc.*
- Yung Yang, *OPP, US EPA*

Technical Advisors to the ICWG

- Michael Luster, *NIOSH*
- Kimber White, *Virginia Commonwealth University*
- Susan Elmore, *NTP*
- Paul Foster, *NTP*
- Dori Germolec, *NTP*

Charge to the ICWG

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

The Process - 1

- NTP provided the ICWG draft “levels of evidence” criteria
  - 5 levels: clear evidence, some evidence, equivocal evidence, no evidence, and inadequate study
- ICWG applied these draft criteria to ~ 30 case studies selected to
  - Provide examples of the kinds of data that could be available
  - Straddle the borders between criteria categories
- Case studies included
  - Results from studies from academia, NTP contract, and other government agencies
  - Results from studies of environmental chemicals and therapeutics
  - Spectrum of the different types of data that could be encountered in immunotoxicology studies
- ICWG assumed cases studies were well-conducted with positive controls giving appropriate responses

Case Study Example

- Slight decrease in body weight gain at high dose only
- Dose-dependent increase in liver weights
- Dose-dependent decrease in spleen weights
- Moderate centrilobular necrosis in the liver at the high dose only
- Minimal decrease in cortical lymphocytes in the thymus
- Minimal decrease in germinal centers in the spleen
- Increased numbers of lymphocytes in the spleen
- Decrease in spleen cell numbers, but increased numbers of Ig+ lymphocytes
- Decrease in antigen-specific IgM and IgG antibody responses
- Increase in delayed hypersensitivity response to KLH
- Decreased NK cell activity
- Decrease macrophage phagocytosis in macrophages obtained from the spleen, but not in macrophages obtained from the peritoneal cavity
- Decreased resistance to bacterial challenge (Listeria and Streptococcus

Issues considered in applying the draft criteria to case studies

- Recovery after exposure to immunotoxicant
- Biological plausibility
- Functional versus non-functional changes
- Inconsistency of changes in immune parameters
- Magnitude and/or biological relevance of changes in immune parameters
- Consistency of dose-responses
- Time-course of changes in immune parameters
- Data gaps that lower the strength of evidence for immunotoxicity
- Immune response enhancement or suppression by toxicants

The Process - 2

- ICWG reviewed the case studies individually and discussed the results as a group
- Revised the draft criteria
- Edited “key points” for consideration in applying the criteria
- Prepared the ICWG report
  - Presentation to the BSC at November meeting for action

Levels of Evidence for Evaluating Immune System Toxicity: level 1

- Clear Evidence of Toxicity to the Immune System
  - Is demonstrated by data that indicate a clear treatment-related (considering the magnitude and the dose-response) effect 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 treatment-related effects on one functional assay and additional endpoints that indicate biological plausibility

Levels of Evidence for Evaluating Immune System Toxicity: level 2

- Some Evidence of Toxicity to the Immune System
  - Is demonstrated by data that indicate a treatment-related effect on one functional parameter with no other supporting data, or
  - Is demonstrated by data that indicate treatment-related changes in multiple non-functional parameters without robust changes in a functional immune parameter or a disease resistance assay, or
  - Is demonstrated by data that indicate non-dose-related effects on functional parameters or a disease resistance assay with other data providing biological plausibility.

Levels of Evidence for Evaluating Immune System Toxicity: level 3

- Equivocal Evidence of Toxicity to the Immune System
  - Is demonstrated by data that indicate non-dose-related effects on functional parameters or a disease resistance assay without other data providing biological plausibility, or
  - Is demonstrated by data that indicate treatment-related changes in a single non-functional parameter without changes in a functional immune parameter or a disease resistance assay, or
  - Is demonstrated by data that indicate immune effects 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: level 4

- No Evidence of Toxicity to the Immune System
  - Is demonstrated by data from studies with appropriate experimental design and conduct that indicate no evidence of biologically relevant changes in immune parameters.

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, treatment-related effects consistent with immunosuppression and immunostimulation will be considered in hazard identification.
- The characterization of immunotoxicity must consider the impact of overt toxicity (e.g., effects on the immune system are not the direct effects of chemical treatment, but are indirect effects mediated via stress and/or other treatment-related responses).
- The characterization of immunotoxicity must consider the intended pharmacology of the chemical. Immunotoxicity is reserved for unintended immunosuppression or immunostimulation.
- It is recognized that recovery may occur following cessation of treatment. However, even transient immune effects that may be observed during treatment or shortly thereafter are important for hazard identification.

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

- 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.
- Functional changes in an immune response should usually be weighted more heavily than non-functional changes.
- Based on historical experience, *in vivo* assays are more sensitive in detecting immunotoxicity than *in vitro* assays. *In vivo* assays also take into account the metabolism of the toxicant that may either reduce or increase immunotoxicity.
- Results in one species or one sex are considered sufficient for evidence of immunotoxicity.
- The purpose of the criteria is for hazard identification only, not risk assessment.

ICWG Comments

- The meeting was a great success
- The ICWG composition was appropriate and included
  - Representatives from academia, industry, and government
  - Experts with knowledge in immunotoxicology, immunotoxicity testing, and/or regulatory needs
- The draft NTP criteria served as a framework for the discussions and for preparing the revised criteria
- Case studies were vital to illustrate how the draft criteria could be applied successfully to experimental data