National Toxicology Program
U.S. Department of Health and Human Services

Explanation of Levels of Evidence for Immune System Toxicity

The NTP describes the results of individual studies of chemical agents and other test articles, and notes the strength of the evidence for conclusions regarding each study. Generally, each study is confined to a single laboratory animal species, although in some instances, multiple species may be investigated under the purview of a single study report. Negative results, in which the study animals do not exhibit evidence of immunotoxicity, do not necessarily imply that a test article is not an immune system toxicant, but only that the test article is not an immune system toxicant under these specific conditions. Positive results demonstrating that a test article causes immunotoxicity in laboratory animals under the conditions of the study are assumed to be relevant to humans, unless data are available which demonstrate otherwise. In addition, such positive effects should be assumed to be primary effects, unless there is clear evidence that they are secondary consequences of overt toxicity to non-immune organ systems.

It is critical to recognize that the "levels of evidence" statements described herein describe only immunologic **hazard**. The actual determination of **risk** to humans requires exposure data that are not considered in these summary statements. This fact is particularly important to keep in mind when communicating study results to the general public.

Five categories of evidence of immune system toxicity are used to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence** and **some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major design or performance flaws **(inadequate study)**. Application of these criteria requires professional judgment by individuals with ample experience with and understanding of the animal models and study designs employed. For each study, conclusion statements are made using one of the following five categories to describe the findings; if warranted, these conclusion statements should be made separately for males and females. These categories refer to the strength of the evidence of the experimental results and not to potency or mechanism.

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.

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.

[1] The term "dose-related" describes any dose-response 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 manifestations at different dose levels or other phenomena.

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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.

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.

When a conclusion statement for a particular study is selected, consideration must be given to key factors that would support the selection of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of immunotoxicity studies in laboratory animals, particularly with respect to the interrelationships between endpoints, impact of the effect on immune function, relative sensitivity of endpoints and specificity of the effect. Factors to consider in selecting the level of evidence of immune system toxicity are given below:

- 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.
- 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.

http://ntp.niehs.nih.gov/go/9399

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