

**Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity  
Battery, Subchronic Inhalation, Subchronic Dermal and  
Immunotoxicity Studies**

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**Office of Pesticide Programs  
U.S. Environmental Protection Agency**



# **Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies**

## **I. Purpose:**

Provide guidance on the weight of the evidence-based (WOE) determination of data needs for neurotoxicity, subchronic inhalation, subchronic, dermal and immunotoxicity studies and provide guidance on how to consider the data needs determination in risk assessment. Evaluation of waiver requests and development of registration review cases are typical situations in which OPP reviewers will make data need determinations.

## **II. Background:**

A determination of data needs for a pesticidal substance should start with the data requirements outlined in 40CFR part 158. Toxicology data requirements are in Subpart C for conventional active ingredients; Subpart U and V for microbial and biochemical active ingredients respectively, and Subpart W for antimicrobial active ingredients. Collectively, these requirements will be referred to as “Part 158 data requirements” throughout this guidance.

Consistent with OPP’s Guiding Principles for Data Requirements, the goals of this document are to ensure there is sufficient information to reliably support registration decisions that are protective of public health and the environment while avoiding the generation and evaluation of data that does not materially influence the scientific certainty of a regulatory decision. It is important to only require data that adequately inform regulatory decision making and thereby avoid unnecessary use of time and resources, data generation costs, and animal testing. Delayed regulatory decisions affect the delivery of health and environmental protections and access to benefits such as pest management tools and safer products. This guidance promotes the full use of existing knowledge to focus on the data needed for a scientifically sound and credible characterization of a specific pesticide’s risk profile for the exposure scenarios of interest and will provide consistency in the determination of toxicology data needs across OPP divisions.

OPP has a long history of practicing flexibility in implementing Part 158 data requirements (§158.30). In particular, as permitted by 40 CFR Part 158.45, waivers can be granted for certain data requirements if found inappropriate, and if there is sufficient data to make the applicable statutory safety determinations. In making decisions regarding data needs, additional data beyond the 158 data requirements may be important to the risk management decision (§158.75), alternative approaches can be accepted, and other data can be used. Most often the toxicity profile of a chemical is based on guideline studies but the database can include non-guideline studies. Data needs decisions are typically case-by-case and consider all existing knowledge including the pesticides’ physical–chemical properties, metabolism/pharmacokinetics, toxicological profile and exposure, available human information, as well as information on structural analogues. In some cases, not all of the “Required” or “Conditionally Required” data may be triggered or needed.

This document specifically provides guidance on appropriate considerations in making determinations on data needs for the neurotoxicity battery (870.6200; acute and subchronic neurotoxicity), subchronic inhalation toxicity (870.3465), subchronic dermal toxicity (870.3250), and the immunotoxicity (870.7800) studies. In addition, guidance on considering the data need determination in the risk assessment is provided. Specifically, to retain a database uncertainty factor (UF<sub>DB</sub>) in the situation where OPP has determined that a study is required (e.g., a waiver of a study is not appropriate) and the study has not yet been conducted.

### **III. Principles for Risk-Based Decisions on Requiring Studies**

#### **1) Neurotoxicity Battery (870.6200)**

The neurotoxicity battery [( *i.e.*, acute (ACN) and subchronic (SCN) studies)] are required for all conventional food and non-food pesticides and are required or conditionally required for antimicrobial pesticides depending on the use pattern. A neurotoxicity study may also be required based on the results of the acute and subchronic studies or other available data. These studies were designed to provide specific endpoints on the central and peripheral nervous systems so that the agency may evaluate the chemical's potential effects on the nervous system. These studies provide data on a wide range of functional tests for evaluating neurotoxicity including sensory effects, neuromuscular effects, learning and memory and histopathology of the nervous system.

When considering the overall toxicity profile for a chemical, there may be no clear signs of neurotoxicity in the conventional studies following acute, subchronic or chronic dosing in multiple species or routes of exposure in the database. To determine the need for data to address neurotoxicity, OPP reviewers should use a WOE approach that takes into account the following evidence:

- (i) ***The Overall Toxicity Profile & Mode of Action (MOA):*** The toxicity profile consists of hazard identification and characterization of all available durations, routes, species, and lifestages. The target organ and adverse outcome(s) are identified. The availability of MOA information enhances the toxicity profile and provides a stronger scientific foundation for entire risk assessment. The availability of metabolism and/or kinetic data which describes the pharmacokinetic (PK) behavior of the chemical, particularly its clearance (slow vs. rapid) and distribution (e.g., to the brain) is particularly relevant to evaluating a chemical's neurotoxic potential. Information on the time course of effects (PK or pharmacodynamics) can also be informative on this point.
- (ii) ***Evidence of Neurotoxicity in the Data base of Toxicology Studies:*** Treatment-related evidence of neurotoxicity from guideline studies or from the open scientific literature includes clinical signs, functional/behavioral effects, brain weight changes and/or neuropathology. It is important to consider whether the observed neurotoxicity is likely due to direct effects on the nervous system or is secondary to general toxicity or toxicity to other organ systems. The presence of a few apparently unrelated or non-specific effects, even though statistically significant, is not necessarily indicative of frank neurotoxicity.

- (iii) ***Evidence of Neurotoxicity in the Database of Toxicology Studies for Structurally-Related Chemicals and/or Those with the same MOA:*** Reviewers consider the profile of pesticides which share the same MOA and/or are in the same chemical class may provide important information with respect to potential neurotoxicity. Specifically, if neurotoxicity data for pesticides which share the same MOA and/or are in the same chemical class suggest more sensitive neurotoxic effects, a neurotoxicity study may be required. All evidence of neurotoxicity for related chemicals is relevant. However, reviewers consider the nature of the findings as they relate to MOA and dose. Secondary effects at very high doses carry less weight than effects which may occur at or near the Point(s) of Departure (PoDs).

**Database Uncertainty Factor (UF<sub>DB</sub>):** If the WOE demonstrates that neurotoxicity studies are needed, then application of a 10x UF<sub>DB</sub> for the lack of both the ACN and the SCN would generally only be **retained only for risk assessment scenarios and relevant durations** that may be impacted by the results of these studies. For example, if an ACN is available but a SCN is not, the UF<sub>DB</sub> would not be relevant for the acute Reference Dose (RfD). However, a UF<sub>DB</sub> for the lack of SCN would be appropriate for repeated exposure scenarios (e.g., short/intermediate/long term). Where the 10x UF<sub>DB</sub> is applied, it would be retained until the required neurotoxicity data are found to satisfy requirements.

If the neurotoxicity battery is required, then OPP encourages the registrants to conduct the acute neurotoxicity study prior to the subchronic neurotoxicity study as the results of the acute neurotoxicity study may aid in dose selection and protocol review for the subchronic study. Moreover, if there is no evidence of neurotoxicity observed in the acute neurotoxicity study then OPP may re-consider granting a waiver for the subchronic neurotoxicity study again considering the available toxicity and metabolism and/or kinetic data. The agency recognizes that due to the particular nature and risk of some pesticides, alternative approaches may be employed to satisfy these data requirements (e.g., comparative cholinesterase study).

## **2) Subchronic (28 or 90-Day) Inhalation Toxicity Study (870.3465)**

A repeated dose inhalation toxicity study is conditionally required (CR) if there is likelihood of significant repeated inhalation exposure to the pesticide as a gas, vapor, or aerosol. Based on estimates of the magnitude and duration of human exposure, studies of shorter duration, e.g., 28 days, may be sufficient to satisfy this requirement. Registrants should consult with the Agency to determine whether studies of shorter duration would meet this requirement.

In the absence of a repeated dose inhalation study, the agency frequently relies on oral toxicity studies to conduct inhalation risk assessments. In December 2009, the agency sought expert advice and input from its FIFRA Scientific Advisory Panel (SAP) on issues related to this route-to-route extrapolation approach in the absence of an inhalation toxicity study (i.e., the use of oral toxicity studies for inhalation risk assessment). Based on the SAP's recommendations in the March 2, 2010 Final Report, the agency has increased its focus on the uncertainties associated with route-to-route extrapolation and is presently considering the need for inhalation toxicity studies more frequently.

To determine the need for additional data to address inhalation toxicity, reviewers should use a WOE approach that builds on considerations developed in 2002<sup>1</sup> and the findings discussed in the 2010 SAP Report<sup>2</sup>. The WOE approach considers all relevant hazard [toxicity, metabolism and/or pharmacokinetics (PK), human data], physical-chemical properties, and exposure (including the margins of exposure (MOEs) from the most recent risk assessment) information as detailed below:

- (i) ***Physical-chemical properties:*** Vapor pressure and Henry's law constant are key considerations with respect to exposure from volatilization of the chemical after sprays have settled as these properties relate to volatility.
- (ii) ***Use pattern and exposure scenarios:*** The degree of inhalation exposure in the form of aerosolized droplets or particles/dusts droplets is influenced by the use pattern and exposure scenarios. The entire array of exposure scenarios should be considered and the scenarios where inhalation exposures are the highest should be identified. Particular consideration should be given to the type of application equipment such as air blast and aerial as well as trigger pump sprays and aerosol can dispensers that are more likely to lead to higher occupational handler inhalation exposure. Particle size of aerosols may be an additional factor to consider for some.
- (iii) ***Margins of Exposure (MOEs):*** MOEs are calculated using a PoD from an oral toxicity study provide benchmarks for risk concerns. Reviewers generally consider MOEs from 10-100X over the Level of Concern (LOC) in combination with other factors such as use pattern, exposure scenarios, exposure data and assumptions used in the MOE calculations to determine overall risk concerns in the absence of data from an inhalation toxicity study. The Level of Concern (LOC) is generally defined by the total uncertainty/extrapolation factors applied. This often includes the 10X factors for interspecies and intraspecies extrapolation and may include other factors such as the FQPA 10X Safety Factor or a database uncertainty factor. This range of 10X-100X is derived from comments from the SAP in the 2010 report that indicate that toxicity resulting from oral exposure is not always a good predictor of toxicity derived from the inhalation route.
- (iv) ***The Overall Toxicity Profile:*** The toxicological profile of the subject chemical and the profile of pesticides which share the same MOA and/or are in the same chemical class may provide important information with respect to potential inhalation toxicity. Specifically, if inhalation toxicity data for pesticides which share the same MOA and/or are in the same chemical class suggest more sensitive inhalation effects, an inhalation toxicity study may be required regardless of MOE. Portal-of-entry irritation potential should also be considered for those chemicals showing irritation type effects via the oral or dermal route as these may also result in inhalation toxicity to the respiratory system. Consideration of the acute toxicity data (eye irritation, dermal irritation, sensitization, corrosivity, acute inhalation toxicity) may also inform the concern for localized repeated inhalation effects and metabolism/pharmacokinetic data may inform the concerns for systemic toxicity concerns.

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<sup>1</sup> SOP 2002.01 — "HED Standard Operating Procedure: "Guidance: Waiver Criteria for Multiple-Exposure Inhalation Toxicity Studies".

<sup>2</sup> <http://www.epa.gov/scipoly/sap/meetings/2009/120109meeting.html>

**Database Uncertainty Factor (UF<sub>DB</sub>):** If the WOE demonstrates that a subchronic inhalation toxicity study is required, then the 10x UF<sub>DB</sub> will be **retained only for inhalation risk assessment scenarios and relevant durations** that may be impacted by the results of this study. Where the 10x UF<sub>DB</sub> is applied, it would generally be retained until such data become available or other factors support reconsideration of the data requirement (e.g., changes to the exposure potential).

### **3) Subchronic (21 or 90 Day) Dermal Toxicity (870.3200; 870.3250)**

The use pattern determines the requirement for a repeated dose dermal toxicity study [ (i.e., Required or Conditionally Required (CR)]. A 21/28-day dermal toxicity study is required for food-use chemicals. The duration of this study is judged to be of adequate duration because higher tiered oral studies (i.e., chronic or carcinogenicity studies) are available which can potentially be used for dermal risk assessments. A 90-day study is required for non-food use chemicals. However, the Agency believes that depending on the toxicological and/or exposure profile of the chemical, the 21/28-day dermal toxicity test may be sufficient in duration. For other chemicals, 21/28-day duration may not be sufficient. For example, professional applicators may be subjected to repeated exposures during the 3 months of peak summer infestations. Since for many pesticides there may be increased toxicity with increased exposure professional applicators may not be adequately protected with a 21/28 day study.

To determine the need for additional data to address dermal toxicity, reviewers should use a WOE approach that considers all relevant hazards (toxicity, metabolism and/or PK, human data), physical-chemical properties, and exposure (including the MOEs from the most recent risk assessment) information as detailed below:

- (i) ***Physical-chemical properties:*** Molecular weight and log Kow (between -1 and +3.5) are considered in the WOE as these properties can aid in predicting those chemicals with high and low potential for dermal absorption. Other properties to consider include: physical state, solubility in water and non-polar solvents, vapor pressure (< 5 mmHg), and boiling point (liquid/solid) >15 C°.
- (ii) ***Use pattern and exposure scenarios:*** Scenarios that result in dermal exposure need to be considered in the WOE analysis for granting a waiver for the dermal toxicity study. This should include the product types (e.g., granular, wettable powder, etc) methods of application, exposure duration (short/ intermediate/long-term), and any potential for post-application exposures.
- (iii) ***Dermal absorption study:*** If an acceptable dermal absorption study is available, a dermal absorption factor (DAF) is derived from that study data. The DAF is used with the oral POD to calculate a dermal equivalent dose (DED). The DED can then be used as the POD in risk assessment *in lieu* of requesting a repeated dose dermal study.

- (iv) **Margins of Exposure (MOEs):** MOEs are calculated using a PoD from an oral toxicity study provide benchmarks for risk concerns. Dermal absorption information can be used if available and sufficient quality. Reviewers generally consider MOEs approximately 10X over the LOC<sup>1</sup> in combination with other factors such as use pattern, exposure scenarios, exposure data and assumptions used in the MOE calculations to determine overall risk concerns in the absence of data from repeated dose dermal toxicity study. Use of oral studies in deriving MOEs for dermal exposure are often overly conservative, As such, the agency can consider a small margin above the LOC (i.e., 10X) compared to the inhalation study discussed above.
- (v) **The Overall Toxicity Profile:** The toxicity profile of the subject chemical and the profile of pesticides which share the same MOA and/or are in the same chemical class may provide important information with respect to potential dermal toxicity. Consideration of the acute toxicity data (eye irritation, dermal irritation, sensitization, corrosivity, acute dermal toxicity) may also inform the concern for repeated dermal effects and metabolism/pharmacokinetic data may inform the concerns for systemic toxicity concerns.

**Database Uncertainty Factor (UF<sub>DB</sub>):** If the WOE does not support granting a waiver of the subchronic dermal toxicity study, then the 10x UF<sub>DB</sub> will be **retained only for dermal risk assessment scenarios and relevant durations** that may be impacted by the results of this study. Where the 10x UF<sub>DB</sub> is applied, it would generally be retained until such data become available or other factors support a reconsideration in the data requirement (e.g., changes to the exposure potential).

#### **4) Immunotoxicity Study (870.7800)**

An immunotoxicity study is required for all conventional and antimicrobial pesticides and is a Tier II, conditionally required, study for biochemical pesticides. The Agency's immunotoxicity study (OPPTS.870.7800) is designed to evaluate the immunosuppressive potential of a chemical by measuring antibody production to sheep red blood cells (SRBCs), a T-dependent antigen, in mice or rats. The T-cell dependent antibody response (TDAR) is dependent on functional T-cells, B-cells and antigen processing/presenting cells and is considered a reliable indicator for measuring humoral immune function. In addition, organ weights, pathological and histopathological examination of the immune system organs and tissues and differential white blood cell counts are typically available from the standard subchronic and chronic standard toxicology studies. In some situations, serum immunoglobulin levels may also be available. However, there is some concern that these endpoints alone may not be adequate to fully characterize the potential for immunotoxicity, as they do not directly evaluate the function of immune components (i.e., humoral or cell-mediated type immunity) which allow a better characterization of potential immunotoxicity of a test chemical.

In 2011, CropLife America (CLA) conducted a retrospective evaluation of 82 immunotoxicity studies to determine the potential regulatory impact of this study. The assessment concluded that the large majority of studies demonstrated no treatment-related effects at the high dose level for the given study. Furthermore, in no case was the immunotoxicity study resulting in the most sensitive endpoint when compared to other existing toxicity endpoints in the data base, indicating the lack of impact of these studies on human health risk assessments. Based on their findings, the CLA requested that the Agency begin granting waivers for the immunotoxicity guideline study based on its authority under

40CFR § 158.45 to grant waivers for studies for which “the data would not be useful in the Agency's evaluation of the risks or benefits of the product.”

In 2012, the Office of Pesticide Programs (OPP) independently conducted a retrospective analysis of over 171 immunotoxicity studies conducted on 155 chemicals (including the 82 chemicals evaluated by CLA) for the purpose of 1) assessing if the results of the guideline immunotoxicity study impact human health risk assessment; 2) determining whether there is sufficient justification for granting waivers for this immunotoxicity study; and 3) proposing a path forward. The Agency's retrospective analysis includes chemicals covering a wide variety of pesticide products and chemical classes. All 155 chemicals were assessed by the T-cell dependent antibody response (TDAR) using the plaque forming assay (P FCA) and/or enzyme linked immunosorbant assay (ELISA) methods. A number of different strains/stocks of mice and/or rats were tested. Out of 155 chemicals tested, 140 were reported to cause no effects on the TDAR up to the highest dose-tested (*i.e.* no evidence of immunotoxicity observed). There were 15 chemicals (10%) that exhibited suppression of anti-SRBC IgM response at relatively high doses. For 9 of these chemicals, immunotoxicity was seen at the same dose or at higher doses at which systemic toxicity occurred in the same study. For the other 6 chemicals, TDAR was seen in the absence of systemic toxicity within the immunotoxicity study. However, for risk assessment purposes, immunotoxicity was not the most sensitive endpoint in the toxicity data base for any of these 6 chemicals. The no-observed-adverse-effect levels (NOAELs) or the lowest-observed-adverse-effect levels (LOAELs) established in all 171 the immunotoxicity studies did not impact the Point of Departure (POD) and toxicity endpoints of concern used for human health risk assessments. The no-observed-adverse-effect levels (NOAELs) or the lowest-observed-adverse-effect levels (LOAELs) established in the immunotoxicity studies did not impact the POD or toxicity city endpoints of concern used for human health risk assessments. Consequently, the immunotoxicity studies would not have been used for risk assessment for all 155 chemicals.

When considering the overall toxicity profile for a chemical, there may be no clear signs of immunotoxicity in the conventional studies following acute, subchronic or chronic dosing in multiple species or routes of exposure in the database. To determine the need for data to address immunotoxicity, reviewers should use a WOE approach that considers all relevant hazard (toxicity, metabolism and/or PK data), physical-chemical properties, and exposure (including the MOEs from the most recent risk assessment) information as detailed below:

(i) ***The Overall Toxicity Profile and Evidence of Immunotoxicity.***

Indicators of potential immune toxicity can be derived from routine measurements and examinations performed in toxicity studies submitted under Part 158 toxicology data requirements. These studies include the subchronic, chronic, carcinogenicity, and reproductive toxicity studies. The immune-related endpoints include: hematology and clinical chemistry parameters, spleen and thymus weights, and histopathological examinations of the spleen thymus and lymph nodes. When immune-related effects are observed it is important to consider whether the observed effects are likely due to direct effects on the immune system or is secondary to general toxicity or toxicity to other organ systems. The presence of a few apparently unrelated or non-specific effects, even though statistically significant, is not necessarily indicative of immunotoxicity. If a substance produces one or more of these primary indicators of immune toxicity, more definitive immunotoxicity testing (870.7800) may be recommended; such decisions will be made on a case-by-case basis.



Potential indicators of immunotoxicity from the Part 158 studies include:

- Hematology Indicators: The typical changes seen in the hematological parameters evaluated in the Part 158 toxicity studies (i.e., hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, Heinz body and methemoglobin formation) are consistent with anemia and/or toxicity to the erythropoietic system rather than the immune system. *However, elevation or depression in white blood cell (WBC) counts; altered differential WBC counts (e.g., lymphocytosis, lymphopenia or eosinophilia) may indicate altered bone marrow function and the potential for decreased production of immune cell precursors or products (e.g., immunoglobulin).*
  
- Clinical Chemistry Indicators: A shift in the albumin/globulin ratio may indicate altered bone marrow function and the potential for decreased production of immune cell response. This change in isolation should not be considered as an indicator.
  
- Organ Weight Indicators: In a toxicity study, non-adaptive organ weight changes provide the first signs of treatment-related changes in target organs during treatment. Since body weight is a co-variant in calculating a relative organ weight, elevated or depressed body weights should be considered when interpreting potential effects on immune organs. Organ weight data alone or in relation to body weights can be sensitive measures of organ atrophy, hyperplasia, or hypertrophy, but yield little information about potential immunotoxic effects particularly as it relates to functional deficits. In general changes in organ weight (elevated or depressed absolute/relative spleen and thymus weights) should not be considered as a standalone measure to determine immunotoxicity because it may also indicate generalized systemic toxicity or indirect immunotoxic effects. In practice, however, changes in organ weights or organ-to-body-weight ratio are more relevant to immunotoxicity when they are associated with appropriate histopathological findings.
  - The thymus is a primary lymphoid organ where most T cells mature and decreased thymus weight may indicate potential immunotoxicity following exposure to immunotoxic chemicals. However, the thymus gland grows rapidly in young animals and begins to involute as the animal reach maturity; therefore it may be difficult to detect and measure thymus weight in adult and very old animals. Also, thymic weight may be highly variable resulting from normal biological variation as well as tissue collection techniques. Evidence of atrophy of the thymus observed in a short-term study (28/90 days) could be an indicator of immunotoxicity. On the other hand atrophy of the thymus in a chronic study may be attributable to the age of the animal rather than to frank immunotoxicity. Therefore, careful considerations should be give changes in the thymus glands in studies resulting from different durations of exposure.
  
  - The spleen is a secondary lymphoid organ where T-cells and B-cells mature. The spleen is also involved in limited erythropoiesis (production of red blood cells), B- and T-cell clonal expansion and serves as a biological filter for circulating blood. Increased spleen weights may result from a variety of causes, including increased hematopoiesis, increased processing of damaged erythrocytes, congestion, and neoplasia. Therefore, careful considerations should be given to isolated findings in the spleen.

- ***Histopathology Indicators:*** The immune system is composed of diverse groups of organs and tissues including those responsible for immune cell hematopoiesis (*e.g.*, bone marrow, spleen), lymphocyte maturation (thymus) and immune surveillance (*e.g.*, lymph nodes). Gross and microscopic examination of lymphoid tissues (*i.e.*, thymus, spleen, lymph nodes) is part of routine toxicity studies. Changes in lymphoid organs histopathology (*e.g.*, thymus atrophy, lymphoid necrosis) may represent either a direct immunotoxic effect or a secondary effect related to a primary non-immunotoxic outcome. Histopathological indicators should thus be evaluated in the context of other findings.
  - Hemosiderin deposits of the spleen may occur secondary to hemorrhage or hemolysis that is unrelated to direct immunotoxicity. Similarly, spleen congestion (passive accumulation of blood) may result from non-immunotoxic effects such as hemolytic anemia or barbiturate euthanasia.
  - Extramedullary hematopoiesis of the spleen is commonly seen in rodents (particularly mice) in the absence of underlying disease and can be indicative of anemia/ hemorrhage/hemolysis, systemic inflammation, splenic trauma/necrosis, decreased hematopoiesis by the bone marrow, and neoplasia. Spleen histopathological changes in the absence of other lesions are unlikely to be specific indicators of immunotoxicity.
  - Histological changes in the thymus, spleen, and lymph nodes at doses lower than those eliciting frank systemic toxicity, on the other hand, may reflect deficits in immune function not typically assessed in other available Part 158 studies. Also, if the test chemical is shown to either stimulate cell proliferation or to cause atrophy and cell depletion in any lymphoid organ without clear evidence for a primary non-immunotoxic etiology, the effect is likely to be viewed as a potential indicator of an immunotoxic effect.

(ii) ***Evidence of Immunotoxicity in the Database of Toxicology Studies for Structurally-Related Chemicals and/or Those with the same MOA:*** All evidence of immunotoxicity for related chemicals is relevant. However, reviewers consider the nature of the findings as they relate to MOA and dose. Secondary effects at very high doses carry less weight than effects which may occur at or near the PoDs. This evaluation should include all relevant information on structural analogs regarding immunotoxicity potential of the chemical class. Results from EPA's Retrospective Analysis may be useful as part of WOE rationale for certain chemical class. Certain classes of chemicals (*e.g.*, the organotins, heavy metals, halogenated hydrocarbons) do not directly target the immune system and therefore would not be expected to be immunotoxic.

**Database Uncertainty Factor (UF<sub>DB</sub>):** If this WOE analysis **does not** indicate a concern for immunotoxicity, then a waiver will be granted for this study and a **UF<sub>DB</sub>** is not warranted. If the WOE does not support granting a waiver of the immunotoxicity study, then the 10x UF<sub>DB</sub> will be **retained only for appropriate risk assessment scenarios and relevant durations.**

- The UF<sub>DB</sub> will not be retained for derivation of the acute reference dose because typically, a high dose may be required to cause immunotoxicity following a single exposure and it is unlikely that a single exposure to a chemical will produce persistent immunotoxicity.
- The UF<sub>DB</sub> will be retained for the short-, intermediate and chronic durations because continuous exposure of at least 28 days is sufficient to induce/ an immunotoxic response (TDAR) and if the test chemical is an immunotoxic compound, then, continuous (short, intermediate and long term) would make the immunotoxicity more severe.

**Alternative Ways to Satisfy the Immunotoxicity Data Requirement:** The agency will be receptive to approaches that effectively incorporate special immunotoxicity endpoints into the battery of routine toxicology studies if it would reduce animal usage while still providing the necessary information within the context of other toxicological endpoints. The Agency recommends that the registrants consult with the Agency in advance and provide specific proposal for inclusion of immunotoxicity endpoints into another guideline study prior to initiation of an alternate study. An applicant or registrant may meet the requirement for immunotoxicity data by:

- Integrating immunotoxicity measures into any of several existing part 158 studies (*e.g.*, 28-day range finding or 90-day subchronic feeding study);
- Conducting an extended One-Generation Reproduction Toxicity study (EOGRTS) *in lieu* of the TDAR guideline study. The immunotoxicity cohort of the EOGRTS (OECD Test Guideline 443) will satisfy the part 158 data requirement.