Immunotoxicity Risk Assessment

Andrew A. Rooney
NIEHS / National Toxicology Program
Office of Health Assessment and Translation
RTP, NC 27540  Andrew.Rooney@nih.gov

April 25 – Risk Assessment Specialty Section Webinar
Objectives and Outline

Objectives

• Present framework for evaluation of immunotoxicity

Outline

• Risk Assessment Basics
• Focus on Hazard Identification
• Weight of Evidence Flow Charts
• Case Studies
  − Mercury
  − Halogenated Pt salts
Problem Formulation

Define Goals

• Define the purpose of the risk assessment
• Why is it being done?

Define Scope and Focus

• Specific or general
• Regulatory framework

Consider Preliminary Knowledge
Hazard Identification

Evaluate the weight of evidence (WOE)

- Human data
- Animal Data

WOE Conclusions for Hazard ID

- Dose-response relationship
- Biological Plausibility
- Causality “Hill Criteria” in epidemiological studies
- Human relevance of animal data
  - Mode of Action (MOA)
Hazard Identification: Data

Obvious Advantage of Human Data

• Quality and availability of exposure data determine utility

Animal Data for Immunotoxicity Risk Assessment

• Animals are good models for assessment of human immunity, although there are differences
• Difference from appropriate control is more important than historical range for evaluation of immunotoxicity
Consideration of Animal Data for Immunotoxicity Risk Assessment

Sex

- Consistency of effects between sexes strengthens WOE
- Lack of consistency does not disprove effect of chemical

Species and Strain

- Data from multiple species or strains strengthens WOE
- Negative data in one species does not disprove effect

Exposure

- **Route** – should match likely human exposure
- **Duration** – should cover half-life of immune component
- **Age at initial exposure** – highly relevant; developmental immunotoxicity can differ qualitatively and quantitatively
Consideration of Animal data for Immunotoxicity Risk Assessment

Local versus Systemic Effects
- Immune function at the site of exposure should be considered in addition to measures of overall immunotoxicity

Reversibility or Irreversibility of Effects
- Early exposure or effects associated with developmental have demonstrated more long lasting effects
- Likelihood of persistence of immune effects depends on timing of exposure, MOA, dose and duration
WOE Approach to Evaluate Database

Applied to single study, endpoint, or database

Harmonized with NTP 2009
“Levels of Evidence for Immune System Toxicity”

• Clear Evidence
• Some Evidence
• Equivocal Evidence
• No Evidence
• Inadequate Study of Immune System Toxicity

Results in one species or sex are sufficient
WOE for Immunotoxicity

Clear Evidence of Toxicity to the Immune System

• Is demonstrated by data that indicate a dose-related 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 indicated biological plausibility
WOE for Immunotoxicity

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
WOE for Immunotoxicity

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
WOE for Immunotoxicity

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.
Weight of Evidence Approach to Hazard Identification

Figures 4.1, 5.1, 7.1

- Organize data
- From most predictive to least
- Flow chart not decision tree
- Answer **ALL** questions
- Use answers to develop Hazard ID conclusions
- Then evaluate other immunotoxicity data

Develop WoE conclusions for immunosuppression hazard ID based on answers to all 7 questions.
Well-controlled clinical and epidemiological studies represent clear evidence of adverse immunosuppression.

**QUESTION 1:** Are there epidemiological studies, clinical studies or case-studies available that provide human data on end-points relevant to immunosuppression (i.e. incidence of infections, response to vaccination, DTH, lymphocyte proliferation, other data)?

Well-controlled clinical and epidemiological studies represent clear evidence of adverse immunosuppression.

GO TO QUESTION #2.
WoE Approach to Hazard ID

Last Step

• Develop conclusions for Hazard ID
• Consider all data (including negative studies)

Identify endpoint or effect with lowest dose for biologically plausible and biologically significant response – critical effect(s)

• May take multiple endpoints forward for hazard characterization or dose-response assessment

Develop WoE conclusions for immunosuppression, immunostimulation, or autoimmunity hazard identification based on answers to ALL questions.
Hazard Characterization

Goal: develop health-based guidance values

- Quantification of Dose-Response
- Dose-response assumed to be non-linear and exhibit a threshold below which effects on the immune system are not expected
- Specific approach depends on purpose and regulatory background identified in Problem Formulation
Dose – Response Output

Health-based guidance values
• Based on regulatory framework (RfD, RfC, ADI, TDI, AEL)

Quantification of effects selected in hazard ID
• Multiple endpoints, studies
• ALL EFFECTS (suppression, stimulation, hypersensitivity, and autoimmunity)

Identify doses associated with lowest effect levels for point of departure (POD)
• Make dosimetric adjustments
• Divide POD by uncertainty factors
Exposure Assessment

Goal: estimate magnitude, frequency, duration, and route of human exposure

• Route of Exposure
  − Relevance of animal data for humans
  − Potential for local immune effects

• Timing of Exposure
  − Potential for developmental immunotoxicity
  − Immunosenescence – effects on elderly
Risk Characterization

Goal: integrate Hazard Identification, Hazard Characterization, and Exposure Assessment

- Present immunotoxicity information and reference values
- Provide an estimate of likelihood that identified adverse effects will occur in exposed people
- Include critical review of the quality of the assessment including uncertainties and confidence in conclusions
- Provide useful synopsis to risk manager
Risk Assessment - Summary

• Risk assessment for immunotoxicity can be performed using the same general approach as other non-cancer health effects

• The WHO/IPCS Guidance for Immunotoxicity Risk Assessment provides background and clear step by step WoE approaches to assess immunotoxicity risk for
  − Immunosuppression
  − Immunostimulation
  − Hypersensitivity, and
  − Autoimmunity
Mercury Case Study

- Illustrates risk assessment guidance for autoimmunity
- Not intended to be a full risk assessment
- Available data
  - Some human data suggest link to autoimmune disease
  - Established animal models of autoimmune disease
Mercury Background

Sources of Exposure

- Natural element – air, water, soil, coal
- 3 Forms – elemental, organic, inorganic
- Principal human exposure
  - Methylmercury (organic)- fish and shellfish
  - Elemental – from amalgam dental fillings

Mercury Toxicity and Health Effects

- Immune
- Nervous system
- Cardiovascular
- Kidney
Weight of Evidence Approach to Hazard Identification for Autoimmunity

- Figure 7.1
- Organize Data
- From most predictive to least
- Flow chart not decision tree
- Answer all 5 questions
- Use answers to develop Hazard ID conclusions
- Then evaluate other immunotoxicity data

**QUESTION 1:** Are epidemiological studies, clinical studies or case-studies available that provide human data on end-points relevant to chemical-induced autoimmunity (i.e. increased incidence of all or specific autoimmune diseases, changes in immune parameters indicative of autoimmunity, increased levels of autoantibodies, decreased regulatory T cell function, evidence of nonspecific stimulation of the immune system, increased levels of markers of inflammation)?

**Targeted epidemiological studies represent the strongest evidence of linkage between chemical exposures and autoimmune disease.**

GO TO QUESTION #2.

**QUESTION 2:** Is there evidence that the chemical causes changes in disease incidence or progression in animal models of autoimmune disease?

**Data from genetically predisposed models may represent clear evidence of disease potential in susceptible individuals.**

GO TO QUESTION #3.

**QUESTION 3:** Is there evidence that the chemical alters immune measures associated with autoimmunity (i.e. autoantibody levels, inflammatory markers, regulatory T cells, lymph node proliferation, etc.) in animal models of autoimmune disease?

**Enhanced measures of self-reactivity and inflammation in animal models of autoimmune disease provide some evidence of autoimmunity.**

GO TO QUESTION #4.

**QUESTION 4:** Is there evidence from general or observational immune assays (lymphocyte phenotyping, cytokines, complement, lymphocyte proliferation, etc.) that the chemical has the potential to modulate autoimmune disease?

**Observational immune assays generally present equivocal evidence for effects on autoimmunity.**

GO TO QUESTION #5.

**QUESTION 5:** Is there histopathological evidence (thymus, etc.) or are there haematological changes that suggest that the chemical causes an immune response against self (i.e. immune complex deposition, inflammatory cell infiltrates)?

**Histopathological evidence may provide supportive evidence for autoimmunity.**

Develop WoE conclusions for autoimmunity hazard identification based on answers to all 5 questions.
WoE Approach to Hazard ID

- Organize data by predictability of assays
- **Human Data**: Strongest evidence

**QUESTION 1**: Are epidemiological studies, clinical studies or case-studies available that provide human data on end-points relevant to chemical-induced autoimmunity? (i.e. increased incidence of all or specific autoimmune diseases, changes in immune parameters indicative of autoimmunity, increased levels of autoantibodies, decreased regulatory T cell function, evidence of nonspecific stimulation of the immune system, increased levels of markers of inflammation)?

Targeted epidemiological studies represent the strongest evidence of linkage between chemical exposures and autoimmune disease.

GO TO QUESTION #2
Question 1 - Human Data

Mercury in autoimmune disease subjects

- Lack of good exposure data – mixed exposures
- Increased OR for rheumatic diseases or lupus with Hg/petroleum or self reported Hg exposure (Dahlgren et al., 2007; Cooper et al., 2004)

Autoimmune elements with Hg-exposure

- Inconsistent data
- Elevated anti-laminin antibodies with mercury vapor exposure; (Lauwerys et al., 1983; Barregard et al., 1997)

Dental amalgam fillings

- Inconsistent, generally negative data
- meta-analysis non significant OR 1.24 (95%CI:0.96,1.61) with development of multiple sclerosis (Aminzadeh and Etminan, 2007)
- Clinical improvement of lupus, thyroiditis, multiple sclerosis on removal of amalgam (Prochazkova et al., 2004; Sterzl et al., 2006)
Question 1 – Human Data summary

✓ Mercury in autoimmune disease subjects
✓ Autoimmune elements with Hg exposure
✓ Amalgam fillings
✓ WoE from epidemiological data:
  • **YES**, database supports association between mercury exposure and autoimmune disease
  • Database lacks a large epidemiological study
  • Quantitation is not possible given lack of dose-response exposure data
Data from genetically predisposed models represent clear evidence of disease potential in susceptible individuals.
Question 2 – Summary of Autoimmune Disease Progression/severity in Animals

✓ YES, induces de novo autoimmune disease in susceptible rodents

✓ YES, accelerates onset and exacerbates severity of autoimmune disease in autoimmune-prone mice

✓ YES, exacerbates severity of disease in mouse models of acquired autoimmunity

✓ Much of the data are from s.c. exposure studies
Enhanced measures of self-reactivity and inflammation in animal models of autoimmune disease provide some evidence of autoimmunity.

QUESTION 3: Is there evidence that the chemical alters immune measures associated with autoimmunity (i.e. autoantibody levels, inflammatory markers, regulatory T cells, lymph node proliferation, etc.) in animal models of autoimmune disease?

GO TO QUESTION #4
Question 3 – Summary of Immune Measures in Autoimmune Disease Models

✓ **YES**, many animal studies report immune parameters associated with autoimmunity:
  - **Autoantibodies** - autoimmune-prone models
  - **PLNA** - Mercury response is well established
  - **Proinflammatory cytokines** – no clear pattern
  - **T-helper cell polarization** - some evidence of Th2 cell bias (associated with autoimmunity and upregulation)

✓ Data suggest mechanism and MOA

✓ Data support exacerbation and acceleration of autoimmune disease in animal models
Observational immune assays generally present equivocal evidence for effects on autoimmunity.

**QUESTION 4:** Is there evidence from general or observational immune assays (lymphocyte phenotyping, cytokines, complement, lymphocyte proliferation, etc.) that the chemical has the potential to modulate autoimmune disease?

Observational immune assays generally present equivocal evidence for effects on autoimmunity.

GO TO QUESTION #5
Question 4 – Summary of General or Observational Immune Data

✓ **YES**, many animal studies report data from observational immune assays that the chemical has the potential to modulate autoimmunity

- Polyclonal B cell activation
- Hypergammaglobulinaemia
- Selective T cell proliferation
- Altered cytokine secretion
- Attenuation of pro-apoptotic signaling, etc.

✓ Data suggest mechanism and MOA
**Question 5 – Histopathology**

- **Histopathological evidence**: equivocal

**QUESTION 5**: Is there histopathological evidence (thymus, etc.) or are there haematological changes that suggest that the chemical causes an immune response against self (i.e. immune complex deposition, inflammatory cell infiltrates)?

Histopathological evidence may provide supportive evidence for autoimmunity.
Question 5 – Summary of Histopathological or Hematological Data

✓ **YES**, many animal studies report supporting histopathological evidence, principally immune complex deposition

✓ Mercury exposure
  - Causes autoantibody formation
  - Results in immunoglobulin deposits in kidneys (renal basement membranes) in rabbits, mice, and rats

✓ Data suggest mechanism and MOA

✓ Data support exacerbation and acceleration of autoimmune disease in animal models
WoE Approach to Hazard ID

- Organize data by predictability of assays
- Identify doses associated with lowest effect levels for point of departure (POD)
  - May involve multiple studies
  - May take multiple endpoints forward as PODs

Develop WoE conclusions for autoimmunity hazard identification based on answers to all 5 questions.

- Then evaluate other forms of Immunotoxicity
Other Forms of Immunotoxicity

**Mercury Immunosuppression**

- Yes – suppressed resistance to infection, NK, macrophage function, antibody production

**Mercury Hypersensitivity and Sensitization**

- Yes – skin and respiratory sensitization data; suggests delayed-type T cell-mediated, not IgE

**Mercury Immunostimulation**

- Some data – increased immunoglobulins, Th2 cell polarization
WoE Conclusions for Hazard ID

Follow the Questions:

1. Human data? YES, not quantitative
2. Disease in autoimmune models? YES, lowest effect levels use for POD
3. Immune markers in autoimmune models? YES, supports disease endpoints, mechanism
4. Observational immune assays? YES, suggest mechanism, support WoE
5. Histopathological data? YES, suggest mechanism, support WoE
Select critical effect and POD (autoimmunity)

- Dose-response: **YES**
- Relevant route of exposure: **YES**, drinking water
- Biological plausibility: **YES**,
- Studies: Hultman and Nielsen, 2001; Nielsen and Hultman, 2002

- Critical Effect: lowest dose for biologically plausible and biologically significant response
  - A. SW mice exposed to HgCl$_2$ for 10 weeks
  - AFA – antifibrallarin autoantibodies
  - Early marker associated with immune complex deposition and renal damage
## POD - Autoimmunity

### Antifibrallarin autoantibodies (AFA) and Mercury accumulation

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dose (mg/l)</th>
<th>AFA positive/total</th>
<th>AFA reciprocal titre</th>
<th>Renal mercury accumulation (µg/g wet weight)</th>
<th>Splenic mercury accumulation (µg/g wet weight)</th>
<th>Whole-body retention (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>0</td>
<td>0/8</td>
<td>—</td>
<td>NR</td>
<td>NR</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0/8</td>
<td>—</td>
<td>0.23</td>
<td>0.009</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2/8</td>
<td>340 ± 424</td>
<td>0.71</td>
<td>0.0232</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8/8</td>
<td>1890 ± 1667</td>
<td>1.63</td>
<td>0.0472</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8/8</td>
<td>4880 ± 2735</td>
<td>3.76</td>
<td>0.120</td>
<td>4.08</td>
</tr>
</tbody>
</table>

\[ \text{NOAEL} = 0.25\, \text{mg/l drinking water} = \text{POD} \]

**Sources:** Hultman and Nielsen, 2001; Nielsen and Hultman, 2002

**Model:** female A.SW mice after drinking water exposure to mercury(II) chloride for 10 weeks
Hazard Characterization

- **NOAEL** = 0.25mg/L in drinking water

- **Dosimetric adjustments**
  - NOAEL = 0.25mg/L in drinking water
  - $\text{NOAEL}_{\text{ADJ}} = 0.25\text{mg/L} \times \frac{0.003\text{L/day}}{0.0221\text{kg weight}}$
  - $\text{NOAEL}_{\text{ADJ}} = 0.0395\text{mg/kg} = 0.04$ rounded = POD

- **POD** = NOAEL$_{\text{ADJ}}$ (could do BMD)

- **POD** = 0.04 mg/kg body weight/day
Hazard Characterization

• **Reference Value** = POD / total UFs
  – POD = 0.04 mg/kg body weight/day

• **Uncertainty Factors** – total = 100
  – Intraspecies UF = 1 **
  – Interspecies UF = 10
  – Subchronic to Chronic UF = 10
  – Database UF = 1

• **Reference Value** = 0.04 mg/kg/100 = 0.0004 mg/kg

**Note autoimmune prone rodents considered good model of susceptible humans; intraspecies UF =1**
Hazard Characterization

• **Reference Value:**
  = 0.0004mg HgCl₂/kg** body weight
  = 0.0003mg Hg²⁺/kg body weight

• **WoE description:**
  − Strong support from animal data and some human data

• **Susceptible populations:**
  − Adjustment not suggested – autoimmune prone mice

• **Susceptible Life stage:**
  − Unknown for autoimmunity

• **Gender:**
  − Females may be at greater risk

**converted from HgCl₂ to Hg²⁺ x 0.739 by weight; *Dose-Response Assessment = Hazard Characterization**
Acknowledgements

WHO/IPCS Guidance Drafting Group

Mercury Case-study authors

• Michael McCabe (University of Rochester)
• Dori Germolec (NIEHS)
• Andrew Rooney (NIEHS)
Halogenated Platinum Salts Case Study

- Illustrates risk assessment guidance for sensitization and allergic response – hypersensitivity
- Focus on respiratory sensitization data
- Not intended to be a full risk assessment
- Available data
  - Large database of occupational studies with sensitization and occupational asthma
  - Some data on sensitization from animal models
Background

Platinum metal use

- Occupational exposure to halogenated Pt salts
  - Pt refineries and catalytic converter plants
- Environmental exposure via car catalysts
  - Little information on speciation
WoE Approach to Hazard ID For Sensitization and Allergic Response

3 Decision Trees to guide risk assessment

- Skin – Figure 6.2A
- Respiratory – Figure 6.2B
- Oral – Figure 6.2C

Figure 6.2A: Decision-tree for the assessment of sensitization and allergic response: skin sensitization.
Is there evidence that the substance is a **respiratory sensitizer** (e.g., data from epidemiological studies, human experience, or laboratory animal studies)?

Is information available on sensitization potency (e.g., BMD or NOEL from an epidemiological or laboratory animal study) available?

Do quantitative risk assessment of **induction** of respiratory sensitization using SAFs to derive acceptable nonsensitizing air concentration; do quantitative exposure assessment, describe risk characterization.

Is sufficient information on respiratory sensitizing potency available to do a semiquantitative risk…

Yes

**Yes**

No

Yes

Yes

No

No
Respiratory Sensitization (not elicitation)

Is there evidence that the substance is a **respiratory sensitiz**er (e.g., data from epidemiological studies, human experience, or laboratory animal studies)?

If no, then do quantitative risk assessment of induction of respiratory sensitization using SAFs to derive acceptable nonsensitizing air concentration; do quantitative exposure assessment, describe risk characterization.

If yes, then is information available on sensitization potency (e.g., BMD or NOEL from an epidemiological or laboratory animal study) available? If no, then do quantitative risk assessment of induction of respiratory sensitization using SAFs to derive acceptable nonsensitizing air concentration; do quantitative exposure assessment, describe risk characterization.

If yes, then do quantitative risk assessment of induction of respiratory sensitization using SAFs to derive acceptable nonsensitizing air concentration; do quantitative exposure assessment, describe risk characterization.
Respiratory Sensitizer – Human Data

• Numerous Case Reports and Occupational Studies

• Symptoms consistent with Pt-specific allergic sensitization
  – **Allergic asthma** (airway constriction, shortness of breath, etc. serious responses may be life threatening)
  – **Rhinitis** (runny nose and sneezing)
  – **Conjunctivitis** (burning and itching eyes)
  – **Urticaria** (hives)
  – **Dermatitis** (itching skin eruptions)

• Few Case Reports include exposure data
Respiratory Sensitizer – Human Data

Epidemiological Studies with Exposure Data

• Baker et al. (1990) and Brooks et al. (1990)
• Bolm-Audorff et al. (1992)
• Linnett and Hughes (1999)
• Merget et al. (2000)
Respiratory Sensitizer – Animal Data

- Few animal inhalation studies of sensitization
- Inadequate to determine exposure-response
  - Inhalation exposure of monkeys to \([\text{NH}_4\text{]}_2\text{PtCl}_6\) produces sensitization\(^1\)
  - Sensitization in 1/8 monkeys exposed to \(\text{H}_2\text{PtCl}_6\) alone
  - Sensitization in 4/8 monkeys exposed to \(\text{H}_2\text{PtCl}_6 + \text{ozone}\)
  - Sensitization in 0/7 monkeys exposed to ozone alone
- Dermal \(\text{Na}_2\text{PtCl}_6\) on mice produces dermal sensitization\(^2\)
- Parenteral exposure of rats and mice to \(\text{Na}_2\text{PtCl}_6\), \([\text{NH}_4\text{]}_2\text{PtCl}_6\), \([\text{NH}_4\text{]}_2\text{PtCl}_4\), and \(\text{K}_2\text{PtCl}_4\) induced sensitization\(^3\)
- Animal data support sensitization from soluble halogenated platinum compounds

\(^{1}\)Biagini et al., 1986
\(^{2}\)Dearman et al., 1998; Schuppe et al., 1997a
\(^{3}\)Schuppe et al., 1997b; Murdoch and Pepys, 1986, 1985, 1984a,b
Other Forms of Immunotoxicity

Immunosuppression

• No data indicating halogenated Pt salts cause immunosuppression

Autoimmunity

• One study (Chen, 2002) disodium hexachloroplatinate induces subcellular changes similar to mercury in mouse strain susceptible to mercury-induced autoimmunity

Immunostimulation

• No data indicating abnormal elevation of cellular or humoral immune function, autoimmunity or allergy in humans or animals
Human Data – Study with NOAEL

Merget et al. (2000)

• 5-year prospective study of workers in a German catalyst plant

• Allergic sensitization indicated by positive Skin Prick Test (SPT) to hexachloroplatinic acid (H₂PtCl₆)

• Exposure categories (high, low, and no exposure) based on job classifications

• Air monitoring data for soluble platinum reported by category
  – Stationary samples from 1992 and 1993
  – Personal samplers from 1993 for high exposure only

• Only study with sufficient exposure and health effects data to perform dose-response analysis
  – LOAEL 52.9 ng/m³
  – NOAEL 3.37 ng/m³
## Merget et al. (2000)

### Exposure Group

<table>
<thead>
<tr>
<th>Exposure Group</th>
<th>Arithmetic Mean$^1$ (ng soluble Pt/m$^3$)</th>
<th>Incidence of Workers with Positive SPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1992</td>
<td>1993</td>
</tr>
<tr>
<td>High</td>
<td>Mean</td>
<td>61.6</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>34.0</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>16</td>
</tr>
<tr>
<td>Low</td>
<td>Mean</td>
<td>6.06</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>8</td>
</tr>
<tr>
<td>No</td>
<td>Mean</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>8</td>
</tr>
</tbody>
</table>

Note: $^1$ Calculated from raw data from Merget et al., 2000
Hazard Characterization

- **NOAEL** = 3.37 ng soluble Pt/m³

- **Dosimetric adjustments**
  - NOAEL = 3.37 ng soluble Pt/m³
  - NOAEL<sub>ADJ</sub> = NOAEL x 10 m³/d / 20 m³/d x 5 d / 7d
  - NOAEL<sub>ADJ</sub> = 1.2 ng soluble Pt/m³

- **POD** = NOAEL<sub>ADJ</sub> (could try BMD)

- **POD** = 1.2 ng soluble Pt/m³
Hazard Characterization

- **Reference Value** = POD / total UF
  - POD = 1.2 ng soluble Pt/m$^3$

- **Uncertainty Factors** – total = 100
  - Intraspecies UF = 10
  - Interspecies UF = 1, human data
  - Time (Subchronic to Chronic) UF = 10
  - Database UF = 1, but could argue for higher value
  - Matrix
  - Use

- **Reference Value** = 1.2 ng soluble Pt/m$^3$ /100
  = 0.012 ng soluble Pt/m$^3$
Hazard Characterization

- **Reference Value:**
  \[= 0.012 \text{ ng soluble Pt/m}^3\]

- **WoE description:**
  - Strong support from human data on sensitization
  - Limited dose-response information and limited exposure data

- **Susceptible populations:**
  - Asthmatics and co-exposure to irritants (ozone, smoking)

- **Susceptible Life stage:**
  - Unknown and data are largely restricted to exposed workers
Weight of Evidence Approach to Hazard ID for Suppression and Stimulation

Figures 4.1, 5.1

• Organize data
• From most predictive to least
• Flow chart not decision tree
• Answer **ALL** questions
• Use answers to develop Hazard ID conclusions
• Then evaluate other immunotoxicity data

**QUESTION 1:** Are there epidemiological studies, clinical studies or case-studies available that provide human data on end-points relevant to immuno-suppression (i.e. incidence of infections, response to vaccination, DTH, lymphocyte proliferation, other data)?

![Flow chart diagram](chart.png)

**QUESTION 2:** Is there evidence that the chemical causes increased incidences of infections and or tumours?

**QUESTION 3:** Is there evidence that the chemical reduces immune function (antibody production, NK cell function, DTH, MLR, CTL, phagocytosis or bacterial killing by monocytes, etc.)?

**QUESTION 4:** Is there evidence from general or observational immune assays (lymphocyte phenotyping, cytokines, complement, lymphocyte proliferation, etc.) that the chemical is immunosuppressive?

**QUESTION 5:** Is there evidence that the chemical reduces haematological changes (e.g. altered WBC counts) suggestive of immune effects?

**QUESTION 6:** Is there histopathological evidence (thymus, spleen, lymph nodes, etc.) that suggests that the chemical causes immunotoxicity?

**QUESTION 7:** Is there evidence that the chemical reduces immune organ weight (thymus, spleen, lymph nodes, etc.)?

Develop WoE conclusions for immunosuppression hazard ID based on answers to all 7 questions.
Weight of Evidence Approach to Hazard Identification for Autoimmunity

Figure 7.1

- Organize Data
- From most predictive to least
- Flow chart not decision tree
- Answer All 5 questions
- Use answers to develop Hazard ID conclusions
- Then evaluate other immunotoxicity data

**QUESTION 1:** Are epidemiological studies, clinical studies or case-studies available that provide human data on end-points relevant to chemical-induced autoimmunity (i.e. increased incidence of all or specific autoimmune diseases, changes in immune parameters indicative of autoimmunity, increased levels of autoantibodies, decreased regulatory T cell function, evidence of nonspecific stimulation of the immune system, increased levels of markers of inflammation)?

**QUESTION 2:** Is there evidence that the chemical alters immune measures associated with autoimmunity (i.e. autoantibody levels, inflammatory markers, regulatory T cells, lymph node proliferation, etc.) in animal models of autoimmune disease?

**QUESTION 3:** Is there evidence that the chemical alters immune measures associated with autoimmunity (i.e. autoantibody levels, inflammatory markers, regulatory T cells, lymph node proliferation, etc.) in animal models of autoimmune disease?

**QUESTION 4:** Is there evidence from general or observational immune assays (lymphocyte phenotyping, cytokines, complement, lymphocyte proliferation, etc.) that the chemical has the potential to modulate autoimmune disease?

**QUESTION 5:** Is there histopathological evidence (thymus, etc.) or are there haematological changes that suggest that the chemical causes an immune response against self (i.e. immune complex deposition, inflammatory cell infiltrates)?

Develop WoE conclusions for autoimmunity hazard identification based on answers to all 5 questions.
WoE Approach to Hazard ID For Sensitization and Allergic Response

3 Decision Trees to guide risk assessment

- Skin – Figure 6.2A
- Respiratory – Figure 6.2B
- Oral – Figure 6.2C

Figure 6.2A: Decision-tree for the assessment of sensitization and allergic response: skin sensitization.
Conclusions

• Risk assessment for immunotoxicity should be performed using the same general approach as other non-cancer health effects

• The WHO/IPCS Guidance for Immunotoxicity Risk Assessment provides background and clear step by step WoE approaches to assess immunotoxicity risk for
  − Immunosuppression
  − Immunostimulation
  − Hypersensitivity, and
  − Autoimmunity
Acknowledgements

WHO/IPCS Guidance Drafting Group

- Nursen Basaran – Hacettepe U.
- Rodney Dietert – Cornell U.
- Dori Germolec – NIEHS
- Peter Griem – Symrise AG
- Geert Houben – TNO
- Robert Luebke – EPA
- Andrew Rooney – NTP
- MaryJane Selgrade – ICF
- Reiko Teshima – NIHS
- Rolaf Van Leewen – RIVM
- Henk van Loveren – RIVM

Representative
- Laura Gribaldo – JRC

Secretariat
- Carolyn Vickers – IPCS/WHO

Case-study authors