Outline

• Evidence from cancer epidemiologic studies on non-Hodgkin lymphoma (NHL) and exposure to trichloroethylene
  – Peer review comments and panel discussion
• Evidence from mechanistic studies
  – Peer review comments and panel discussion
• Integration of human and mechanistic data
• Panel discussion and vote on the NTP preliminary level of evidence conclusion for NHL
NHL: Background information

• Relatively uncommon with high survival:

<table>
<thead>
<tr>
<th>US rates (per 100,000)*</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>23.9</td>
<td>16.3</td>
</tr>
<tr>
<td>Mortality</td>
<td>8.2</td>
<td>5.1</td>
</tr>
</tbody>
</table>

– 5-year survival rate: approx. 70%

• Risk factors:

– Occupational (limited evidence): [bold]benzene[/bold], ethylene oxide, 2,3,7,8-TCDD, polychlorinated biphenyls, [bold]phenoxy herbicides[/bold], styrene, [bold]ionizing radiation[/bold], possibly [bold]chlorinated solvents or other organic solvents[/bold]

– Non-occupational: smoking (follicular lymphoma only), viral infections, immunosuppressive disorders and drugs, certain autoimmune diseases, chemotherapy drugs

*Data: NCI SEER 2006-10
**Agents in [bold]reported in one or more studies included in evaluation
## Studies of NHL and TCE: Cohort and nested case-control studies (10)

<table>
<thead>
<tr>
<th>Population</th>
<th>Studies</th>
<th>Exposure assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nordic (occupational populations) (3)</td>
<td>Hansen 2013</td>
<td>Urine TCA and job</td>
</tr>
<tr>
<td></td>
<td>Vlaanderen 2013</td>
<td>JEM and census linkage</td>
</tr>
<tr>
<td></td>
<td>Raaschou-Nielsen 2003</td>
<td>Blue collar workers in TCE companies</td>
</tr>
<tr>
<td></td>
<td>Lipworth 2011</td>
<td></td>
</tr>
<tr>
<td>US aircraft/aerospace (degreasing) (4)</td>
<td>Radican 2008</td>
<td>Qualitative or semi-quantitative JEM</td>
</tr>
<tr>
<td></td>
<td>Boice 2006</td>
<td>based on work histories</td>
</tr>
<tr>
<td></td>
<td>Morgan 1998</td>
<td></td>
</tr>
<tr>
<td>Other US (electronics mfg, uranium workers, environmental) (3)</td>
<td>Bahr 2011</td>
<td>Qualitative JEM</td>
</tr>
<tr>
<td></td>
<td>Silver 2014</td>
<td>Work history link to dept.-year exposure matrix</td>
</tr>
<tr>
<td></td>
<td>Bove 2014</td>
<td>Estimated cumulative TCE in water by residence</td>
</tr>
</tbody>
</table>

JEM = job-exposure matrix
# Studies of NHL and related subtypes: Case-control studies (7)

<table>
<thead>
<tr>
<th>Population</th>
<th>Studies</th>
<th>Exposure assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe and US populations</td>
<td>Cocco 2013 (pooled)</td>
<td>Detailed semi-quantitative JEM, multiple metrics</td>
</tr>
<tr>
<td>US population (women)</td>
<td>Deng 2013/Wang 2009</td>
<td>Generic JEM</td>
</tr>
<tr>
<td>Montreal population</td>
<td>Christensen 2013</td>
<td>Semi-quantitative JEM</td>
</tr>
<tr>
<td>Nordic populations</td>
<td>Persson-Fredriksson 1999</td>
<td>Self-reported ranked exposure</td>
</tr>
<tr>
<td></td>
<td>Hardell 1994</td>
<td>Self-reported exposure; work histories</td>
</tr>
</tbody>
</table>

JEM = job-exposure matrix

- 2 meta-analyses (Scott-Jinot 2011, Karami 2013)
### Study quality: NHL

**Study Quality** | **Studies**
--- | ---
High | Cocco 2013
Moderate | Hansen 2013, Radican 2008

- Most studies of low or low to moderate quality and with limited sensitivity to detect associations
- One study had potential bias that would likely overestimate risk
- Two studies had other methodological concerns
# NHL: Most informative studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design/population</th>
<th>Strengths/limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocco et al. 2013</td>
<td>Pooled case-control study Europe; 4 constituent studies</td>
<td>Large study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Semi-quantitative exposure assessment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exposure-response relationships</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Detailed assessment NHL and subtypes</td>
</tr>
<tr>
<td>Hansen et al. 2013</td>
<td>Pooled and updated cohort incidence Nordic pop.; various occupations</td>
<td>Moderately large study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biomonitoring</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Misclassification of exposure a concern for levels of exposure</td>
</tr>
<tr>
<td>Radican et al. 2008</td>
<td>Cohort mortality US aircraft workers</td>
<td>Moderately large study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Semi-quantitative exposure assessment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Confounding by co-exposures cannot be ruled out</td>
</tr>
</tbody>
</table>

Light grey = high quality; mid-grey = moderate quality
Limited evidence of an association between NHL or related subtypes and exposure to TCE

- Moderately elevated risks observed in several studies with different study designs and in different populations, but the strength of the evidence varies across studies.
- Relatively strong association and positive exposure-response trends in the most informative (InterLymph) study (Cocco et al. 2013) and its component study (Purdue et al. 2011).
- Meta-analyses suggest statistically significant increased risk for NHL across studies.

Limitations
- Lack of strong association and exposure-response relationships in cohort studies.
- Some case-control studies had methodological limitations.
- Confounding by co-exposure to chlorinated or organic solvents cannot be ruled out in some (e.g., aircraft workers) studies.
NHL ever-exposed to TCE by study quality: Consistent findings of modest increase in several studies

<table>
<thead>
<tr>
<th>Study Quality</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High</strong></td>
<td></td>
</tr>
<tr>
<td>Cocco 2013</td>
<td>1.40 (0.92-2.14)</td>
</tr>
<tr>
<td><strong>Moderate</strong></td>
<td></td>
</tr>
<tr>
<td>Hansen 2013</td>
<td>1.26 (0.90-1.76)</td>
</tr>
<tr>
<td>Radican 2008</td>
<td>1.36 (0.77-2.40)</td>
</tr>
<tr>
<td><strong>Low to Low/Moderate (bias null)</strong></td>
<td></td>
</tr>
<tr>
<td>Silver 2014</td>
<td>0.87 (0.57-1.34)</td>
</tr>
<tr>
<td>Christensen 2013</td>
<td>1.20 (0.50-2.89)</td>
</tr>
<tr>
<td>Bahr 2011</td>
<td>0.99 (0.40-2.46)</td>
</tr>
<tr>
<td>Lipworth 2011</td>
<td>1.31 (0.98-1.75)</td>
</tr>
<tr>
<td>Wang 2009</td>
<td>1.20 (0.85-1.70)</td>
</tr>
<tr>
<td>Boice 2006</td>
<td>0.21 (0.02-2.28)</td>
</tr>
<tr>
<td>Raaschou-Nielsen 2003</td>
<td>1.50 (1.16-1.94)</td>
</tr>
<tr>
<td>Persson &amp; Fredrikson 1999</td>
<td>1.20 (0.55-2.63)</td>
</tr>
<tr>
<td>Morgan 1998</td>
<td>1.36 (0.35-5.25)</td>
</tr>
<tr>
<td><strong>Low (bias positive)</strong></td>
<td></td>
</tr>
<tr>
<td>Hardell 1994</td>
<td>7.20 (4.01-12.94)</td>
</tr>
</tbody>
</table>
Positive exposure-response for multiple exposure metrics in the most informative study

<table>
<thead>
<tr>
<th>Exposure metric</th>
<th>NHL (all types)</th>
<th>Follicular cell</th>
<th>CLL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>InterLymph (Cocco et al. 2003): High probability of exposure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High intensity &gt; 75 ppm</td>
<td>2.2 (0.7–6.7)*</td>
<td>1.5 (0.2–13)*</td>
<td>3.2 (0.6–18)*</td>
</tr>
<tr>
<td>Duration (years)</td>
<td>$P_{trend} = 0.009$</td>
<td>$P_{trend} = 0.028$</td>
<td>$P_{trend} = 0.010$</td>
</tr>
</tbody>
</table>

**NCI- SEER (Purdue et al. 2011) (Component study)**

| Average weekly exposure   |                          |                 |              |
| Per 99 ppm-hr/wk          | $P_{trend} = 0.02$       | $P_{trend} = 0.005$ | $P_{trend} = 0.16$ |
|                           | 1.11 (1.02–1.21)*        | 1.15 (1.04–1.28)* | 1.09 (0.96–1.24)* |
| Cumulative                | $P_{trend} = 0.08$       | $P_{trend} = 0.01$ | $P_{trend} = 0.16$ |
| Per 65,520 ppm-hr         | 1.10 (0.99–1.22)*        | 1.17 (1.04–1.32)* | 1.11 (0.96–1.27)* |

Fisher test for combined probability (probability, duration, frequency, and intensity)
$P = 0.004$ NHL, 0.015 follicular cell, and 0.005 CLL (Cocco et al. 2013)

* Adjusted OR (95% CI)
Increased risk of NHL across studies in meta-analyses

<table>
<thead>
<tr>
<th>Exposure category</th>
<th>Scott &amp; Jinot 2011 mRR (95% CI) # studies</th>
<th>Karami et al. 2013 mRR (95% CI) # studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever exposure</td>
<td>1.23 (1.07-1.42) 17</td>
<td>1.32 (1.14-1.54) 19</td>
</tr>
<tr>
<td>Highest exposure</td>
<td>1.43 (1.13-1.82) 13</td>
<td>NR</td>
</tr>
</tbody>
</table>

Low sensitivity to removal of individual studies or selection of alternative RRs (Scott & Jinot 2011)

Low to moderate heterogeneity

Some evidence of publication bias
NHL human cancer studies: Reviewer questions

• Comment on whether the scientific information from the cancer studies in humans for TCE is clear, technically correct, and objectively presented.

• Provide any scientific criticisms of NTP’s NHL cancer assessment of the epidemiologic studies of exposure to TCE, including how the findings from the individual studies were interpreted and the evidence across studies was synthesized.

• Identify any information that should be added or deleted.
Mechanistic data for NHL

- TCE is associated with lymphomas in both humans and experimental animals
  - Seen in female mice from TCE inhalation
- Little is known about the mechanisms of NHL: Most are B cells
- Immunomodulation is a strong risk factor for B cell NHL
  - Antigenic stimulation is proposed as a potential mode of action (MOA) for immunomodulation-induced NHL, but other MOAs may be possible
- TCE immune effects have been evaluated in both humans and animals
  - Measured immune biomarkers
  - No studies directly evaluated immunomodulation as a MOA for TCE-associated NHL
- Informational group focused on whether TCE-induced immune effects were consistent with the proposed MOA for cancer
Immunomodulation is linked to cancer including NHL

- Evidence comes from studies of patients with immunosuppression associated diseases or autoimmune diseases

- Immunosuppression-associated diseases or conditions
  - Organ transplant patients
    - Stopping immunosuppressant therapy causes partial to complete regression
  - HIV patients
  - Genetically immunodeficient patients
Autoimmune diseases and NHL risk

Rheumatoid arthritis

- US
- Sweden
- CA, US Men
- CA, US Women
- Sweden
- UK
- Denmark
- Sweden
- Finland
- Italy
- Sweden
- Germany
- US

Scleroderma

- Sweden
- Detroit
- US
- Denmark
- Meta-analysis

Systemic lupus erythematosus

- US
- Korea
- Taiwan
- Denmark
- International
- Denmark/Sweden
- InterLymph
- Denmark
- Sweden
- Italy
- Sweden
- International
- US

Sjogren’s Syndrome

- US
- Spain
- Taiwan Men
- Taiwan Women
- Norway
- Meta-analysis (11)
- Denmark
- Sweden
- InterLymph
- Denmark
- Finland
- France

Meta-analysis
Proposed MOA for NHL induction: Antigen-induced B cell activation

- Only B cells undergo somatic mutation after they mature
- Antigen stimulation increases the risk of mutation
- Other immunomodulatory models may exist

![Diagram showing the proposed mechanism of antigen-induced B cell activation in NHL induction.](image-url)
Human immunomodulation studies

Types of studies
• Prospective cohort study
• Case-control studies
  • Individual
  • Pooled
• Cross-sectional studies
  • Occupational
  • Population

Endpoints measured
• Scleroderma
• B cell numbers
• B cell activity
• Antibody isotypes
• Autoantibodies
• Cytokines
• Viral reactivation
Evidence of TCE-induced immunomodulation in human studies

- Autoimmune diseases (scleroderma)
- Peripheral blood lymphocytes
  - Inconclusive cytokine changes
  - B cell activation
  - Antibodies
- Autoantibodies
  - In patients with hypersensitive skin disorder
- Herpes virus reactivation

Red = Detected increase
Blue = Detected decrease
Green = Detected increase and decrease
Experimental animal immunomodulation studies

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Species</th>
<th>Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichloroethylene</td>
<td>Mouse*</td>
<td>Protein adducts</td>
</tr>
<tr>
<td>Metabolites</td>
<td>Rat</td>
<td>Anti-adduct antibodies</td>
</tr>
<tr>
<td></td>
<td>Guinea pig</td>
<td>Autoantibodies</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>Cytokines</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leukocyte number</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leukocyte activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacterial infection</td>
</tr>
</tbody>
</table>

*Mostly MRL\(^{+/+}\) mice which spontaneously develop a lupus-like autoimmunity
TCE-induced immunomodulation in experimental animal studies

TCE

Lipid peroxidation

Protein adducts

B cell activation

Anti-adduct antibodies

Anti-self antibodies

Peripheral leukocytes

Bacteria fighting immune cells

Death

Persistent infection

Autoimmune hepatitis

= Suspected step
Red = Detected increase
Blue = Detected decrease
TCE-induced B cell-activity and proposed MOA

**Consistent**
- Increased autoimmune disease
- Increased autoantibodies
- Increased anti-adduct antibodies
- Increased IgG
  - Experimental animals
- Increased microbial infection/reactivation

**Inconsistent**
- Decrease in B cell numbers
- Decrease/no effect in B cell activation
- Decreased IgG, IgM
  - Human
Limitation of mechanistic data

- Few human studies looked at TCE-induced immunomodulation
- Most experimental animal studies used a transgenic mouse model (MRL\(^{+/+}\))
- Endpoints in experimental animals don’t match those in humans
- No studies looked at immunomodulation and cancer
- B cell activity endpoints are inconsistent
Mechanistic data: Summary

- Immunomodulation is strongly linked to NHL
- TCE cause immunomodulation in humans and experimental animals
  - Evidence for autoimmunity
  - Some evidence for immunosuppression
- The available studies were not able to provide convincing evidence that TCE causes NHL via the proposed immunomodulatory MOA
  - No studies were designed to test hypothesis
  - Might be other MOAs
- Overall, TCE-induced immunomodulation resulting in NHL is biologically plausible
NHL mechanistic studies: Reviewer questions

- Comment on whether the mechanistic data for NHL are clear, technically correct, and objectively presented.
- Provide any scientific criticisms of the NTP’s interpretation and application of the mechanistic data for assessing effects of TCE.
- Identify any information that should be added or deleted.
NHL: Integration of evidence

- Epidemiology studies provide limited evidence of an association between exposure to TCE and NHL in humans
- TCE causes lymphoma in experimental animals
- Toxicological/mechanistic evidence for TCE-induced immunomodulation leading to NHL is biologically plausible, but not conclusive
Preliminary level of evidence conclusion

- Limited evidence of a causal association between exposure to trichloroethylene and NHL from studies in humans

Reviewer questions

- Comment on the overall cancer evaluation for NHL and whether the available data support NTP’s preliminary level of evidence conclusion.

- Provide any scientific criticism of the NHL overall assessment and integration of the human cancer and mechanistic data.

- Vote on whether the science information supports NTP preliminary level of evidence for NHL.