

# TCE and T Cell Activation



Neil R. Pumford, Ph.D.

University of Arkansas

Kathleen G. Gilbert, Ph.D.

University of Arkansas for  
Medical Sciences

# Autoimmunity

- Loss of self-tolerance that results in immune reactions against one's own or self antigens.
- Etiology appears to multifactorial:
  - Genetic Factors
  - Environmental Factors
    - Chemicals
    - Microbial infections

# Autoimmunity

- **Over 80 separate autoimmune diseases including:**
  - **Hasimoto's thyroiditis**
  - **Type 1 diabetes mellitus**
  - **Rheumatoid arthritis**
  - **Multiple sclerosis**
  - **Systemic sclerosis (scleroderma)**
  - **Systemic lupus erythematosus**

# Autoimmunity

- **Autoimmune diseases effects over 9 million Americans (1 in 5)**

# Trichloroethylene

?

- Does trichloroethylene cause or exacerbate an autoimmune response?
- Mechanism?

**Autoimmune Disease  
Inflammation and Fibrosis**

# Trichloroethylene Case Reports

- Over 100 case reports associating trichloroethylene with autoimmune diseases
- Systemic sclerosis (scleroderma) and systemic lupus erythematosus

# Trichloroethylene and Autoimmune Disease

- Byers et al., 1988
  - Altered ratios of T lymphocyte subpopulations
  - increased incidence of auto-antibodies
- Kilburn and Warshaw, 1992
  - Associated with lupus erythematosus and ANA
- Clark et al., 1994
  - Association of perceived exposure to solvents including trichloroethylene with ANA

# Trichloroethylene and Autoimmune Disease

- Nietert et al., 1998
  - Occupational exposure was associated with an increased risk of systemic sclerosis
- Garabrant et al., 2003
  - Associated with systemic sclerosis but not significant



# Experimental Design

Autoimmune-Prone  
MRL+/+ Mice



Trichloroethylene  
(TCE) in drinking water

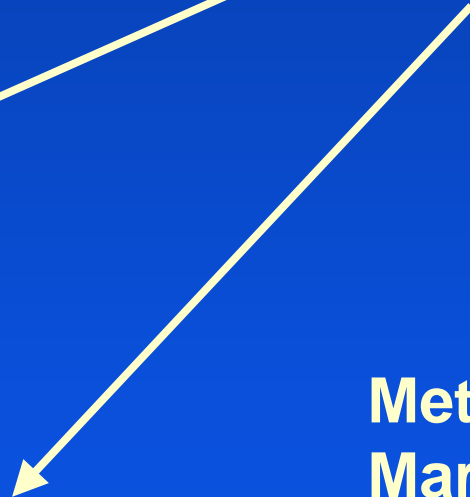


Serum  
Spleen & lymph nodes  
Liver & lungs

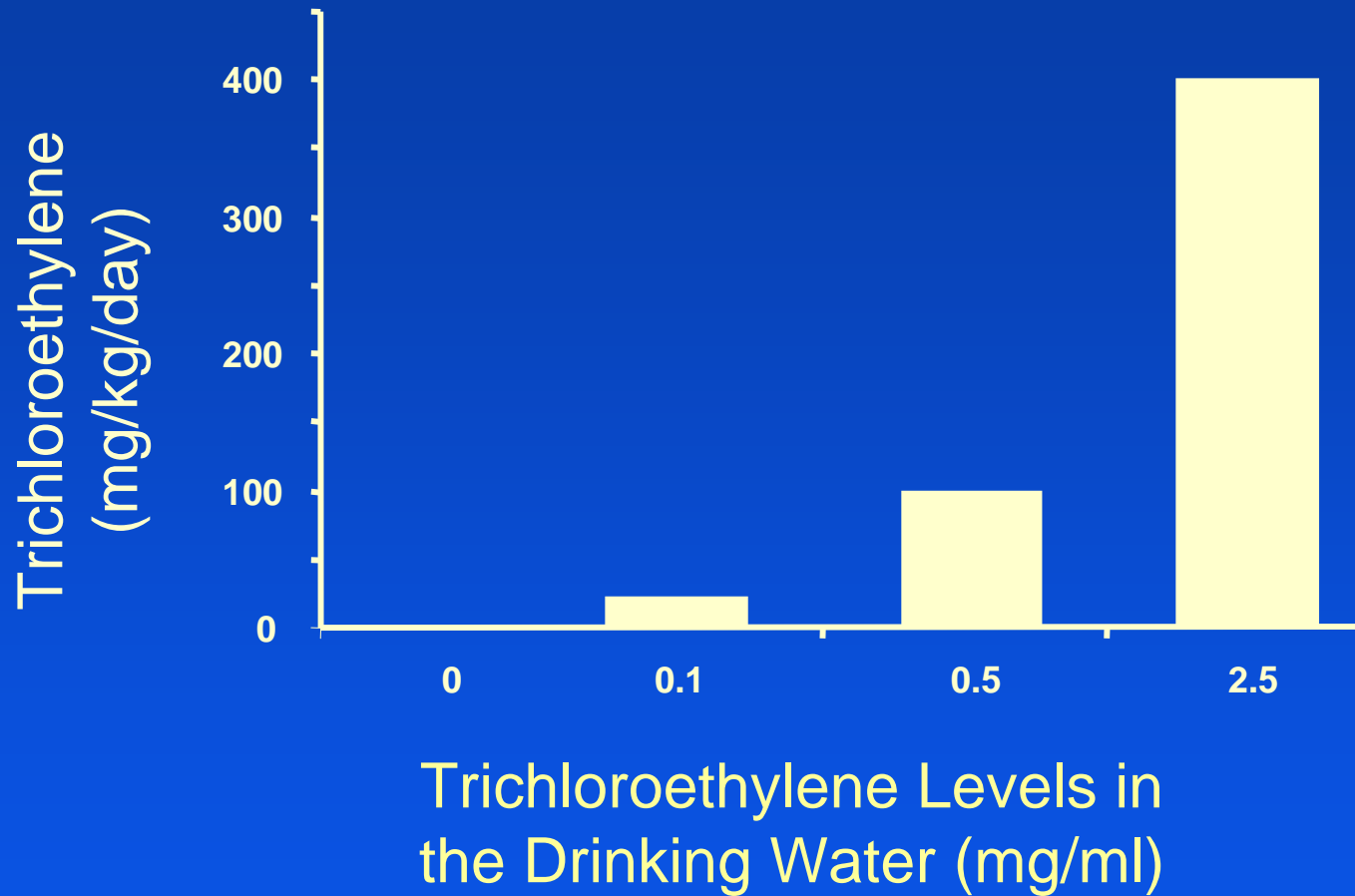
Toxicity  
Autoimmune markers

T-cell Activation  
Cytokines (IFN $\gamma$  & IL-4)

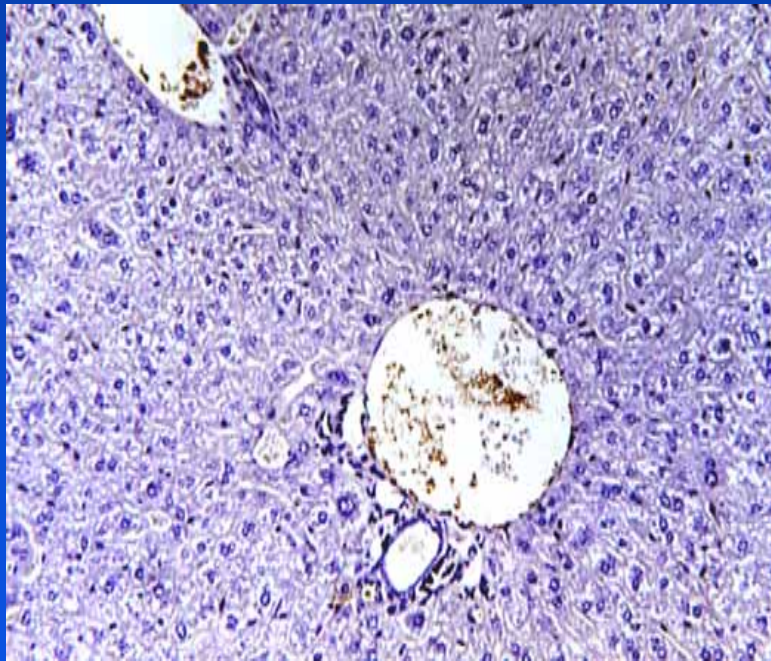
Metabolic Activation  
Markers of Oxidative stress



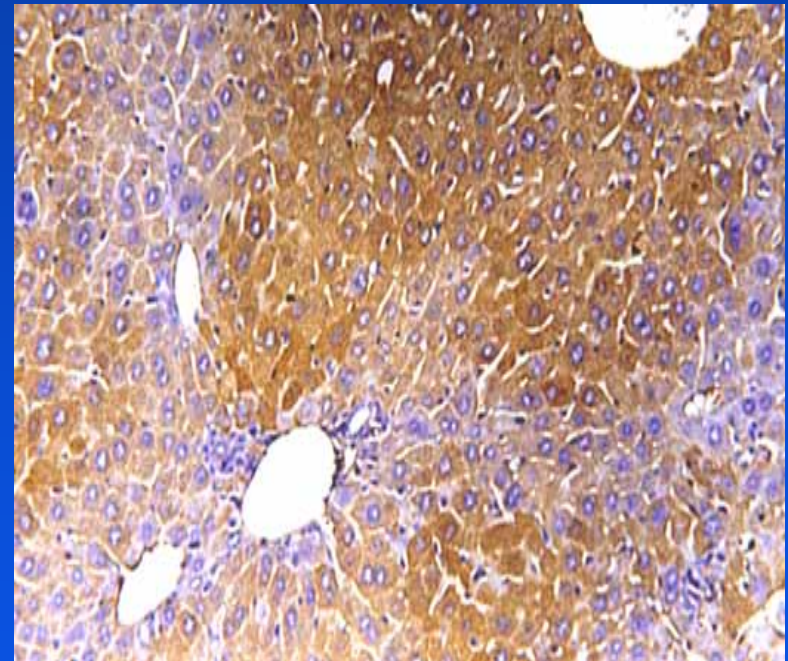
# Low-Dose Chronic Study



# Immunohistochemistry for Trichloroethylene-Protein Adducts



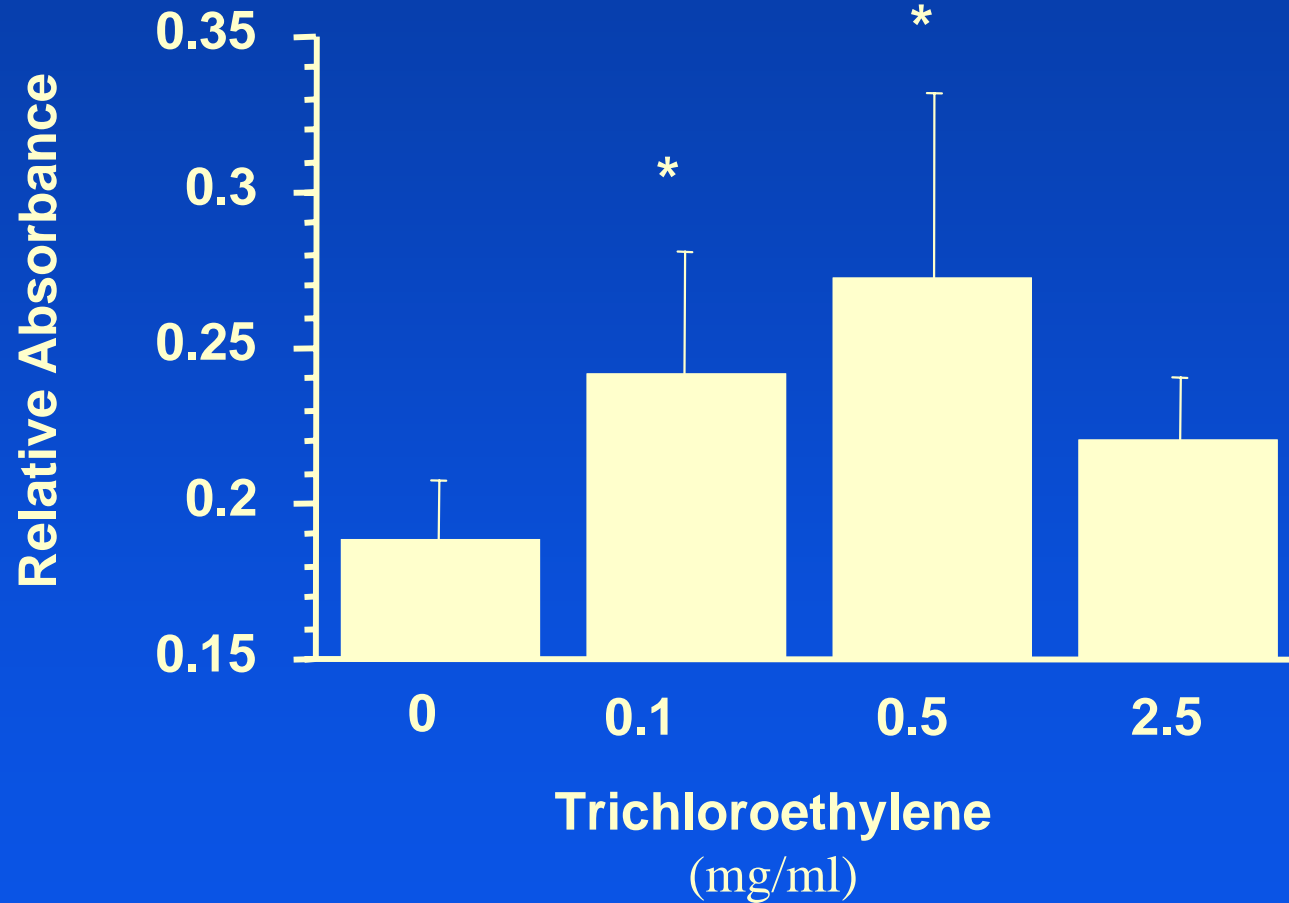
Control





Trichloroethylene

# Antinuclear Antibodies

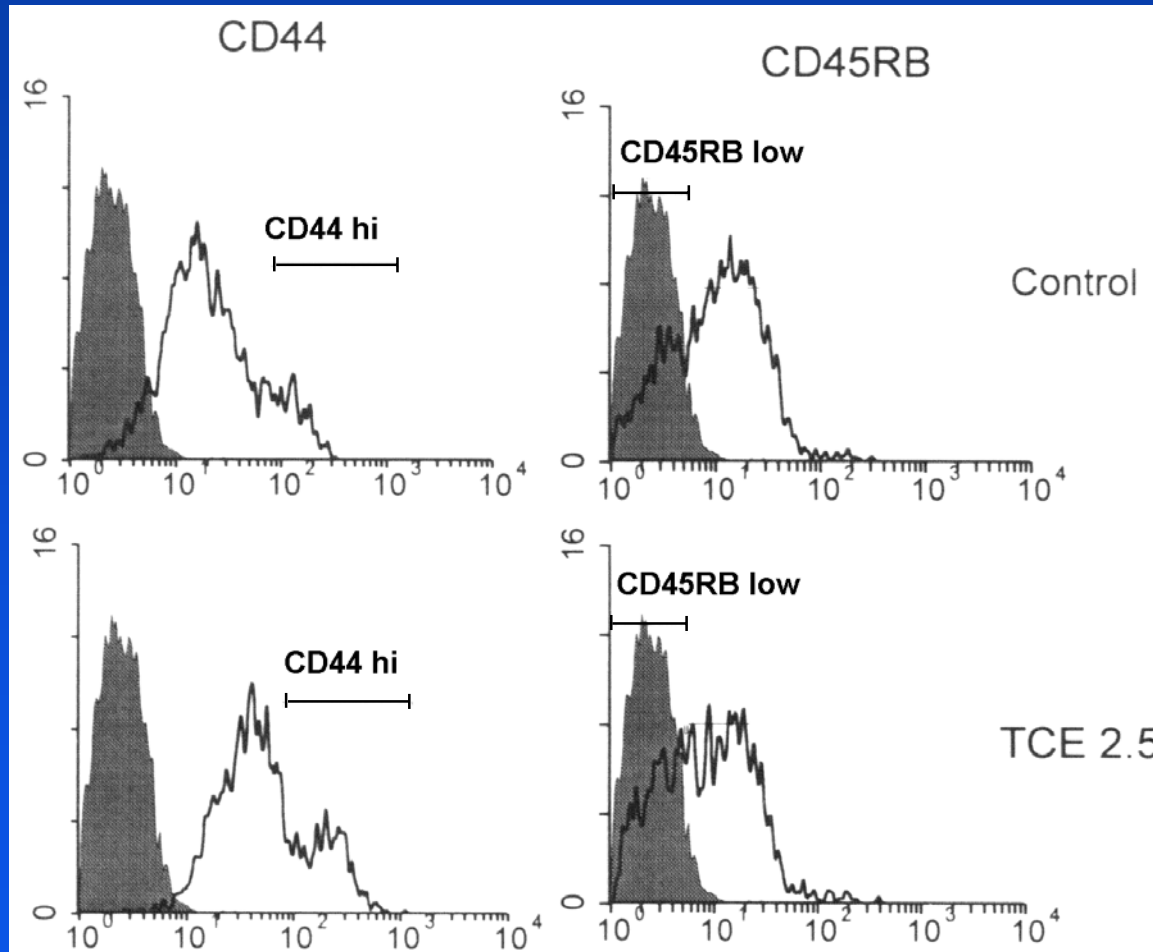
4 Weeks



# Activation of CD4+ T Cells

-  CD44 expression on CD4<sup>+</sup> T cells has been used to monitor the transition from naïve to effector state
-  CD45RB is often used as a second marker of T cell activation

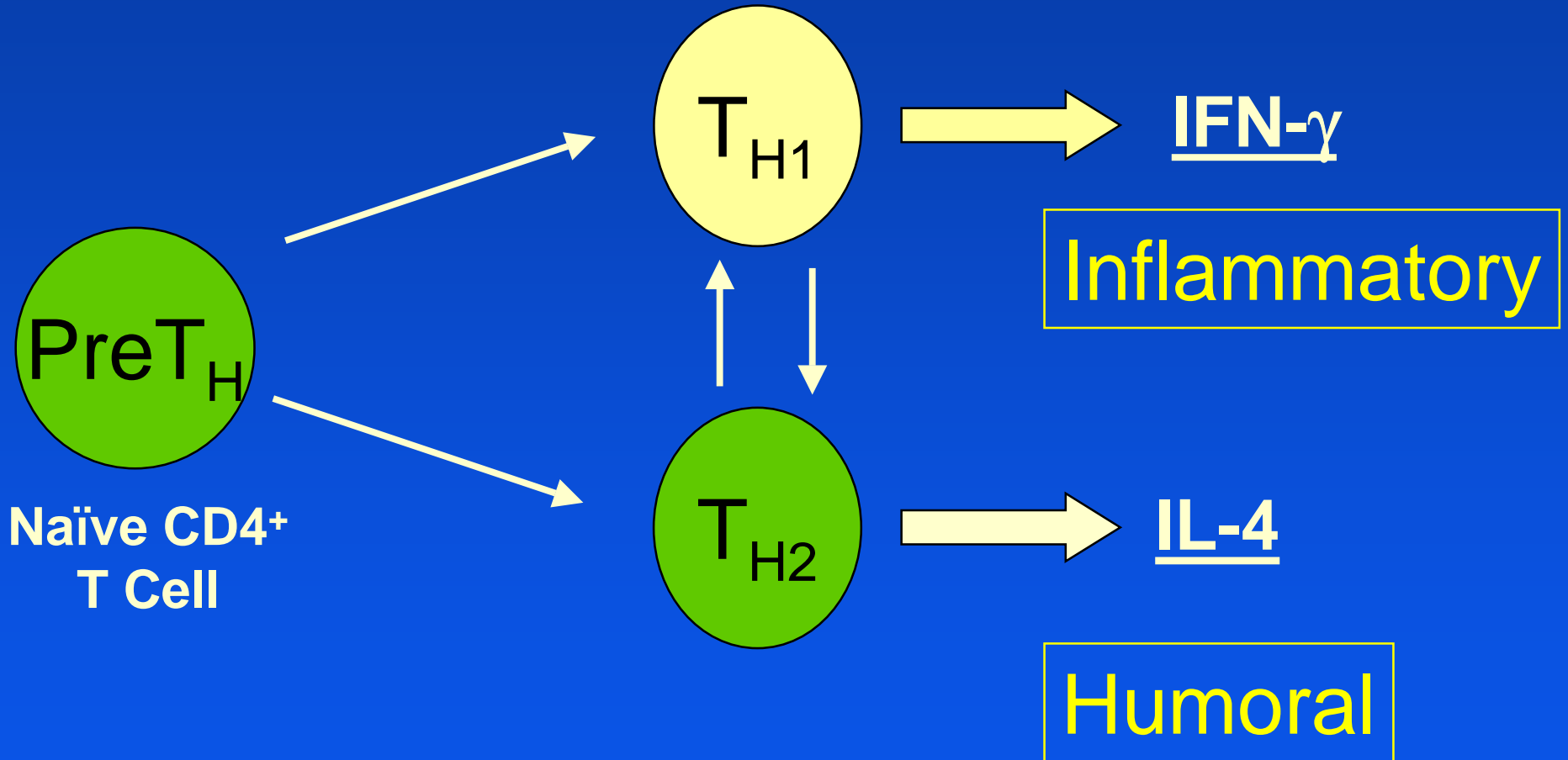
# Expression of CD44 and CD45RB from Mice Treated for 4 Weeks with Trichloroethylene



# CD4<sup>+</sup> T Cell Activation in Spleens following treatment with Trichloroethylene

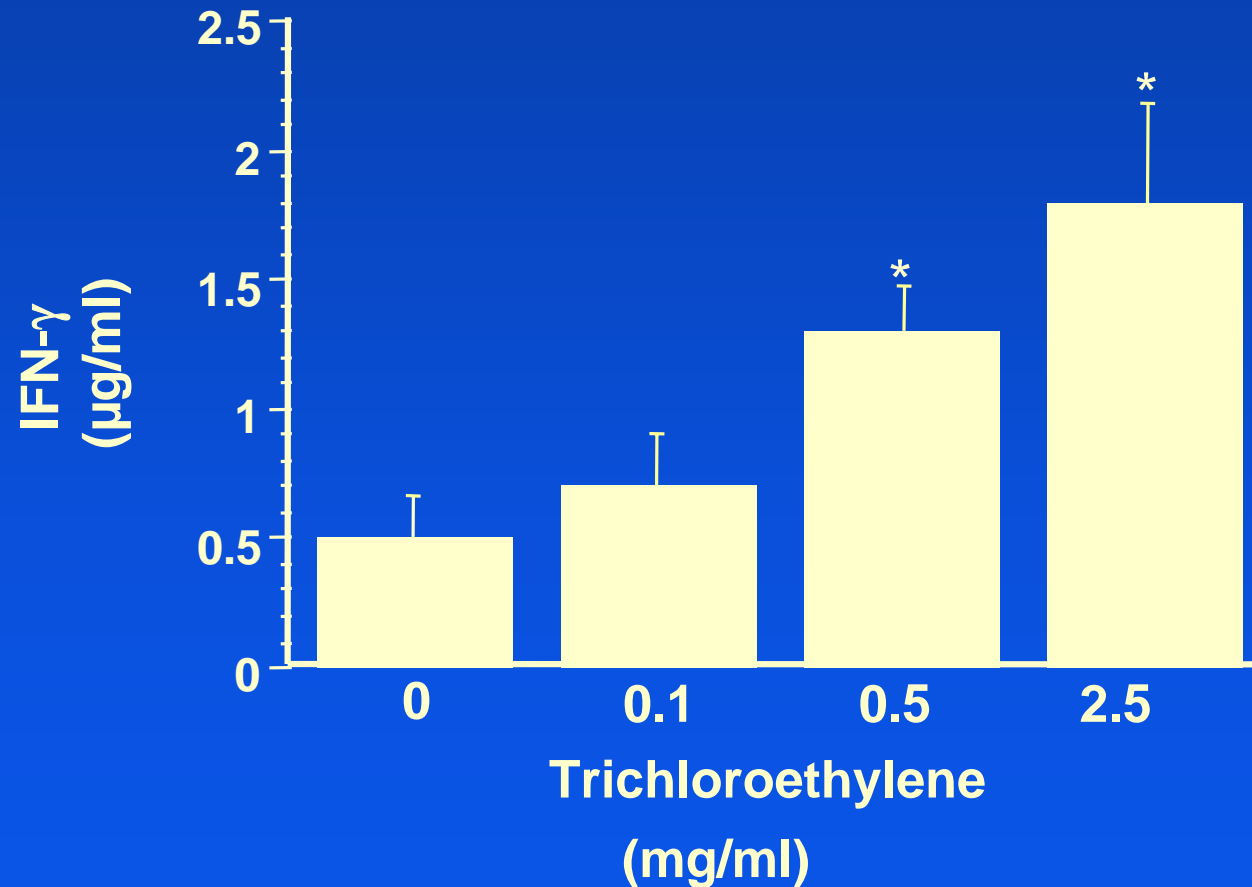
	<b>CD44<sup>hi</sup></b> % Total Cells	<b>CD45RB<sup>low</sup></b> % Total Cells
<b>Control</b>	<b>39</b>	<b>58</b>
<b>0.1 TCE</b>	<b>39</b>	<b>59</b>
<b>0.5 TCE</b>	<b>44</b>	<b>64</b>
<b>2.5 TCE</b>	<b>53</b>	<b>69</b>

# CD4<sup>+</sup> T Cell Maturation

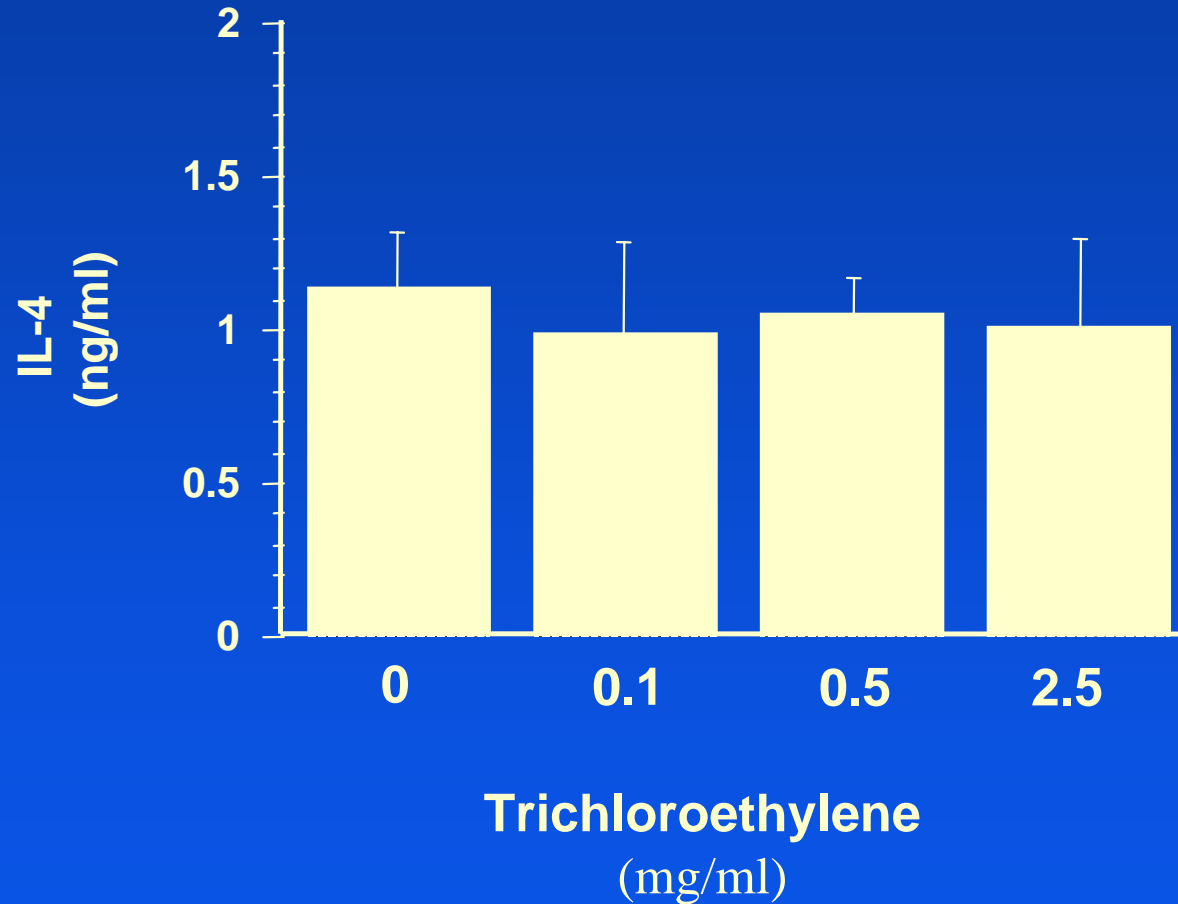




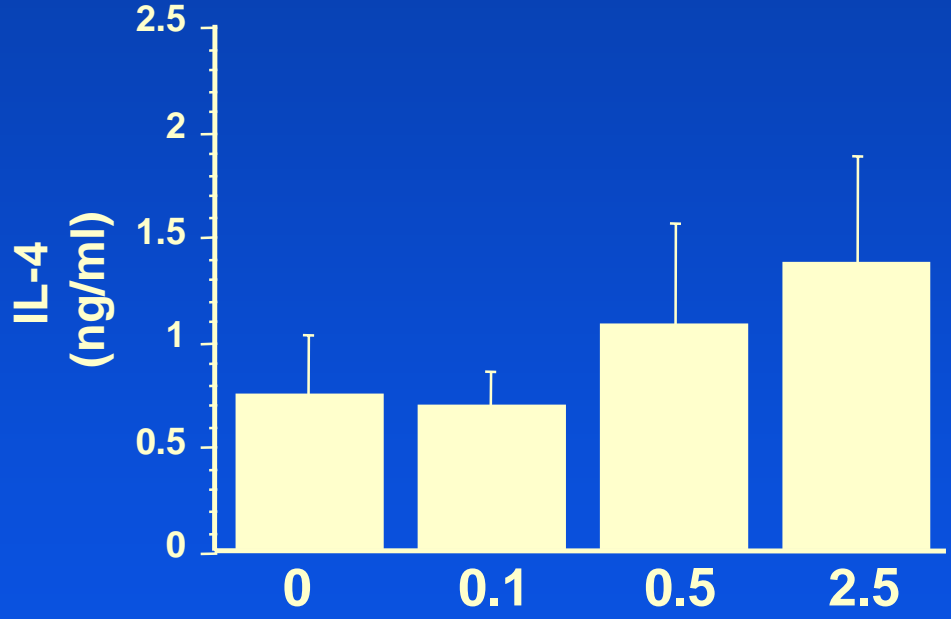
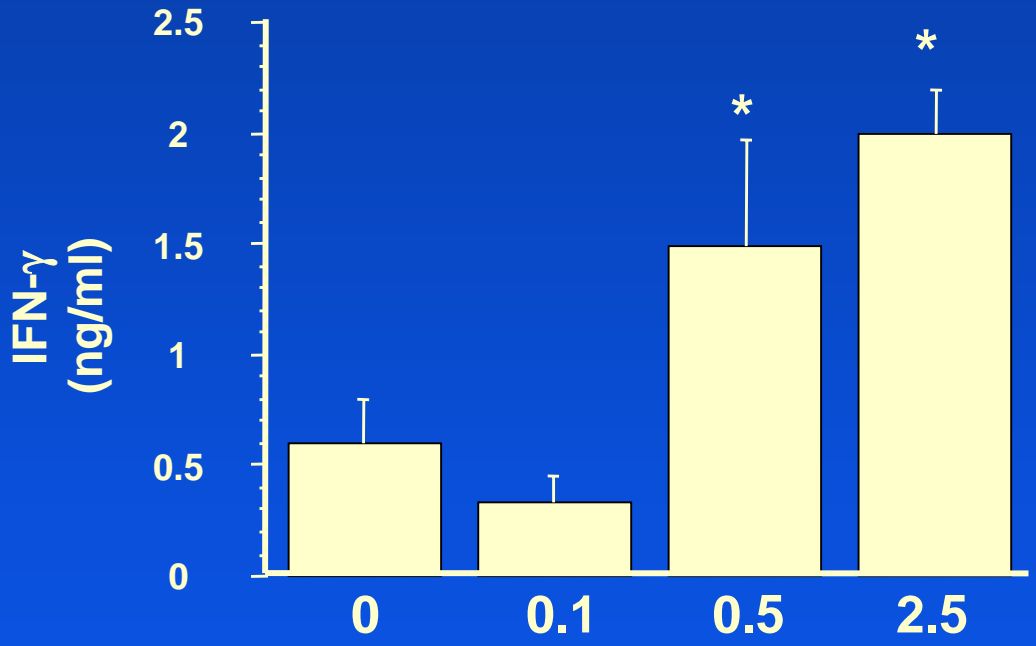
# Interferon- $\gamma$ Levels in MRL $+/+$ Mice (4 weeks)



# IL-4 Levels in MRL<sup>+/+</sup> Mice (4 weeks)



# Cytokine Levels (32 weeks)

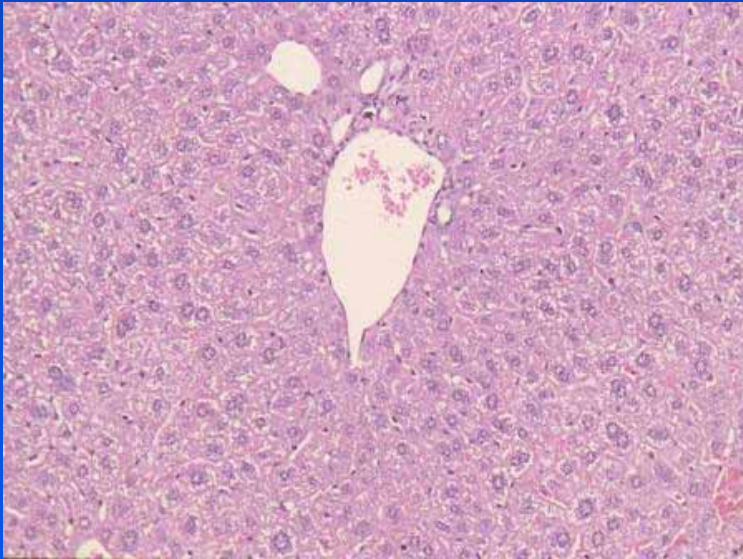


Trichloroethylene  
(mg/ml)

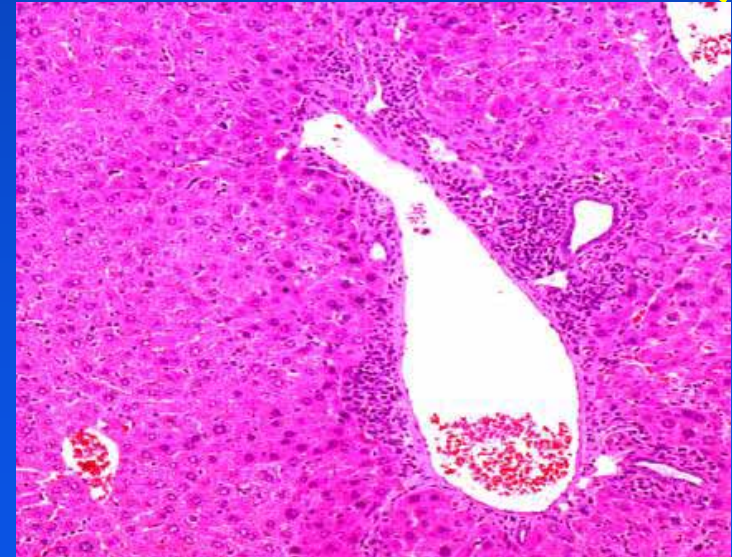
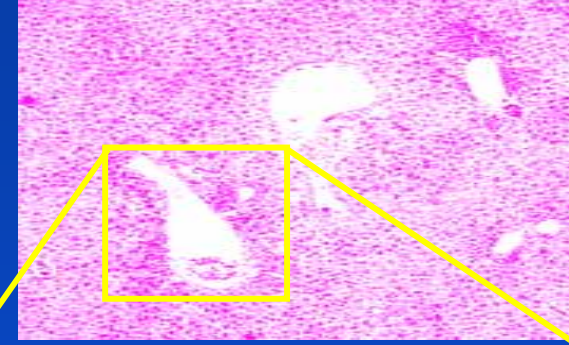
# Toxicity Testing

- Mild, but significant increase in serum ALT levels indicating hepatic damage.

# Liver Histology

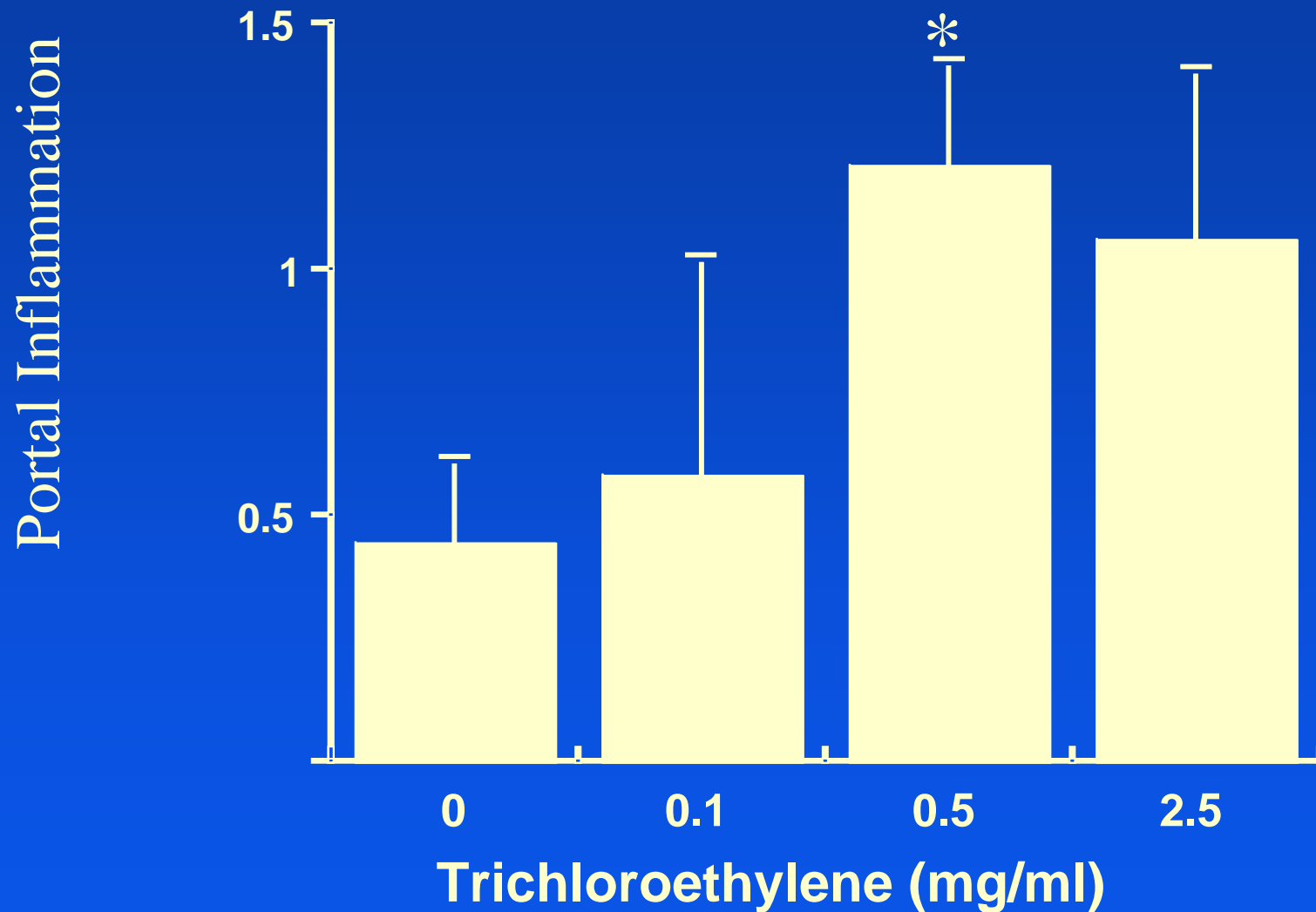


**Control**

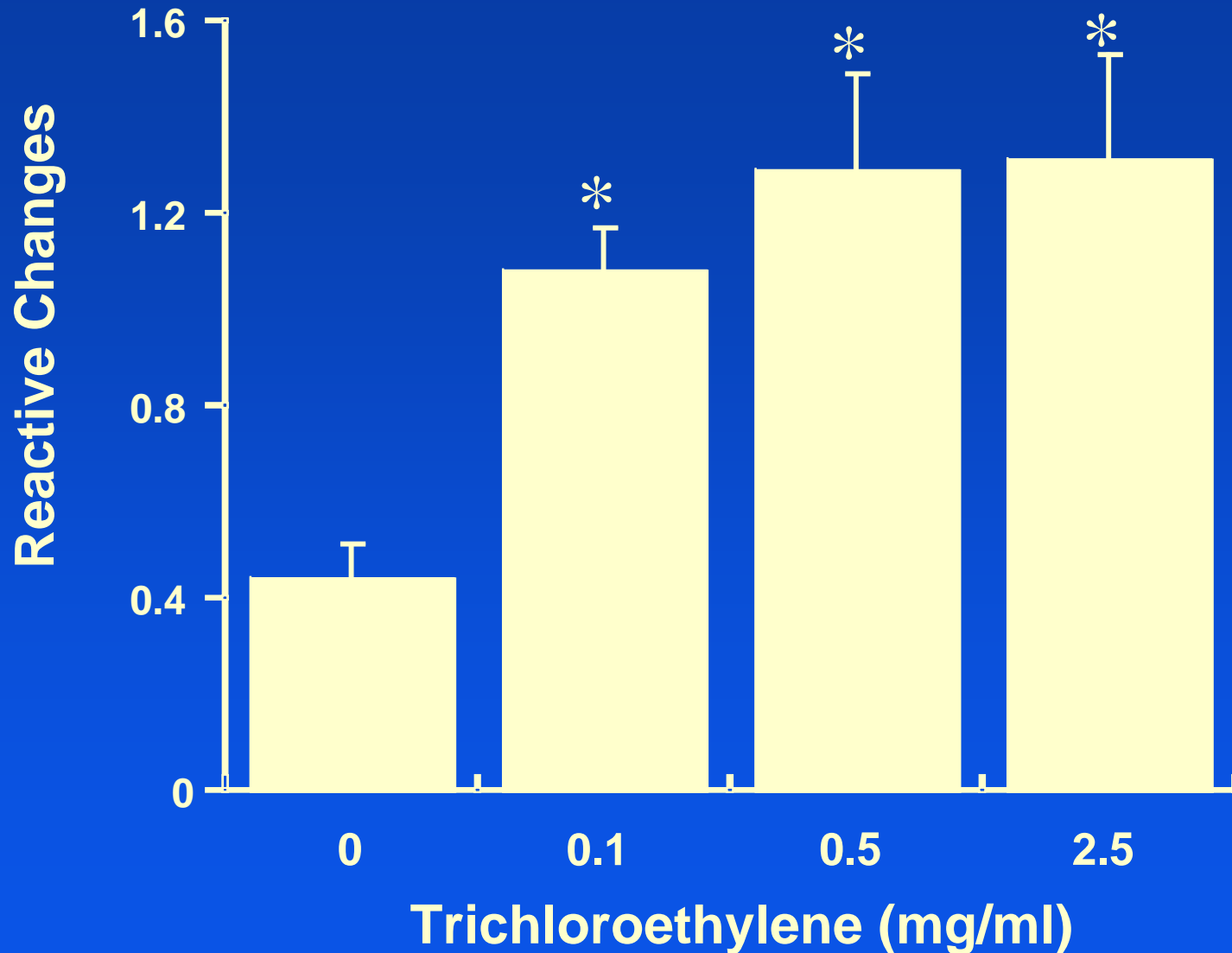


**TCE**

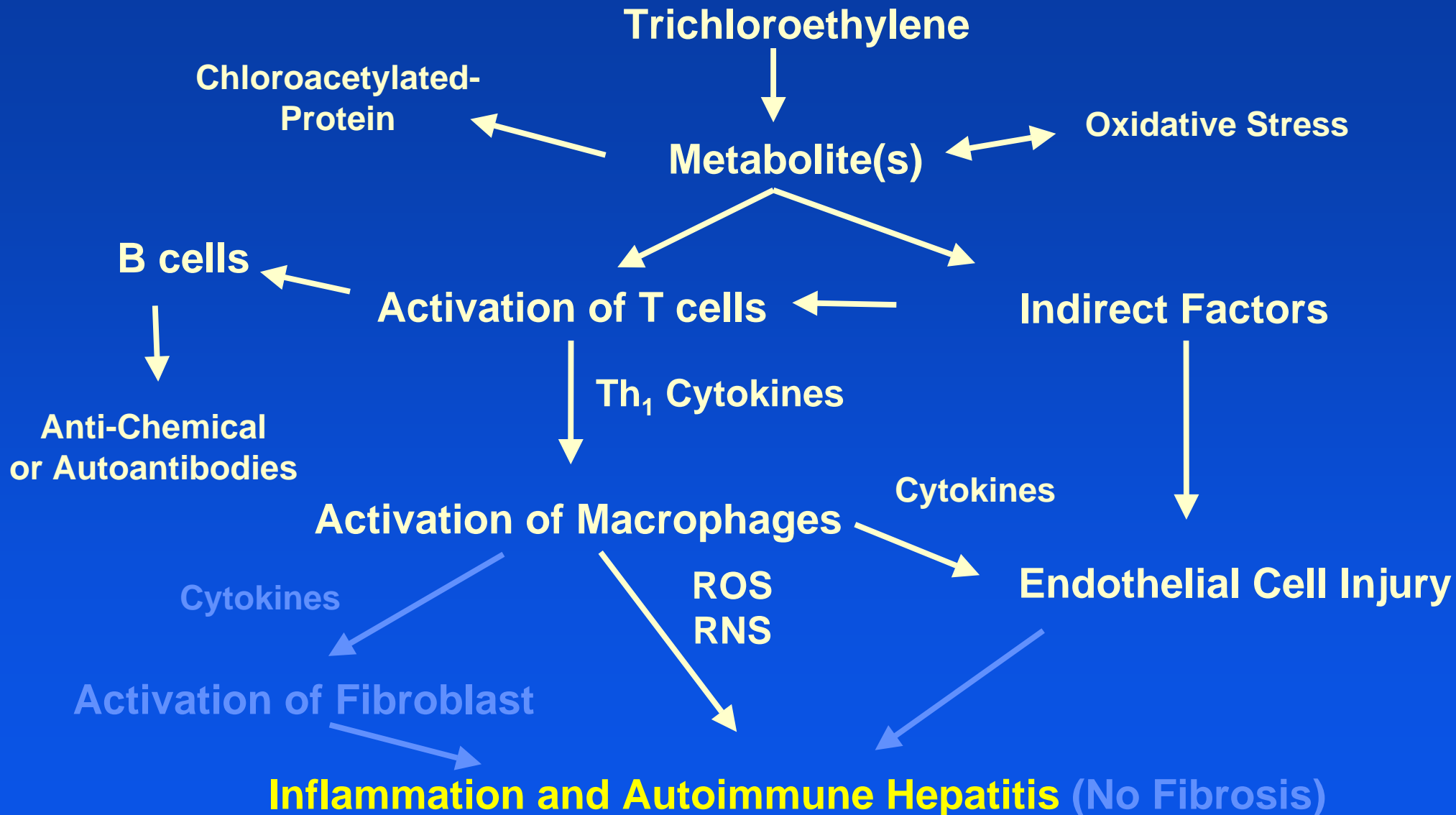
# Liver Histopathological Score



# Liver Histopathological Score

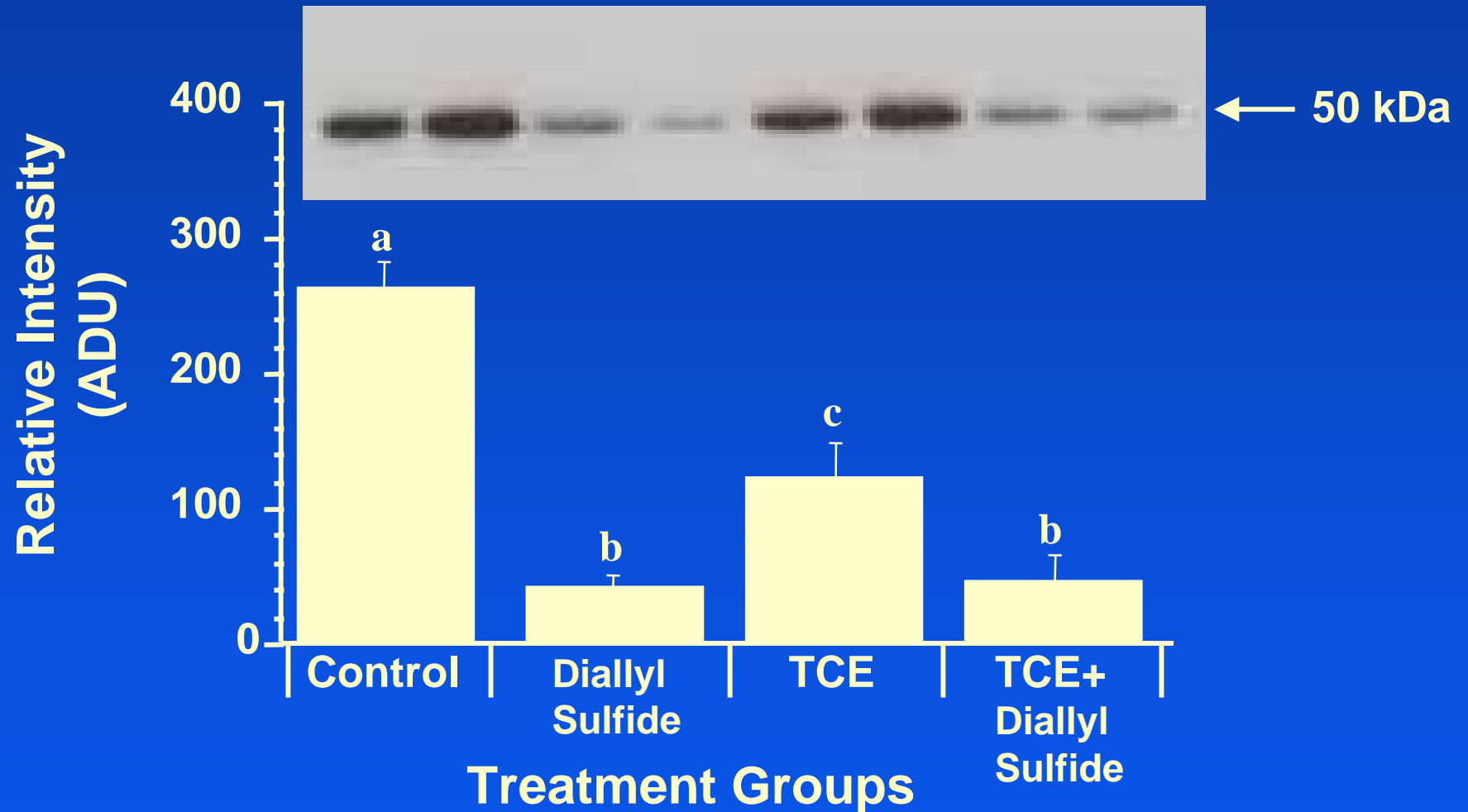


# Mechanism of Trichloroethylene-Induced Autoimmunity

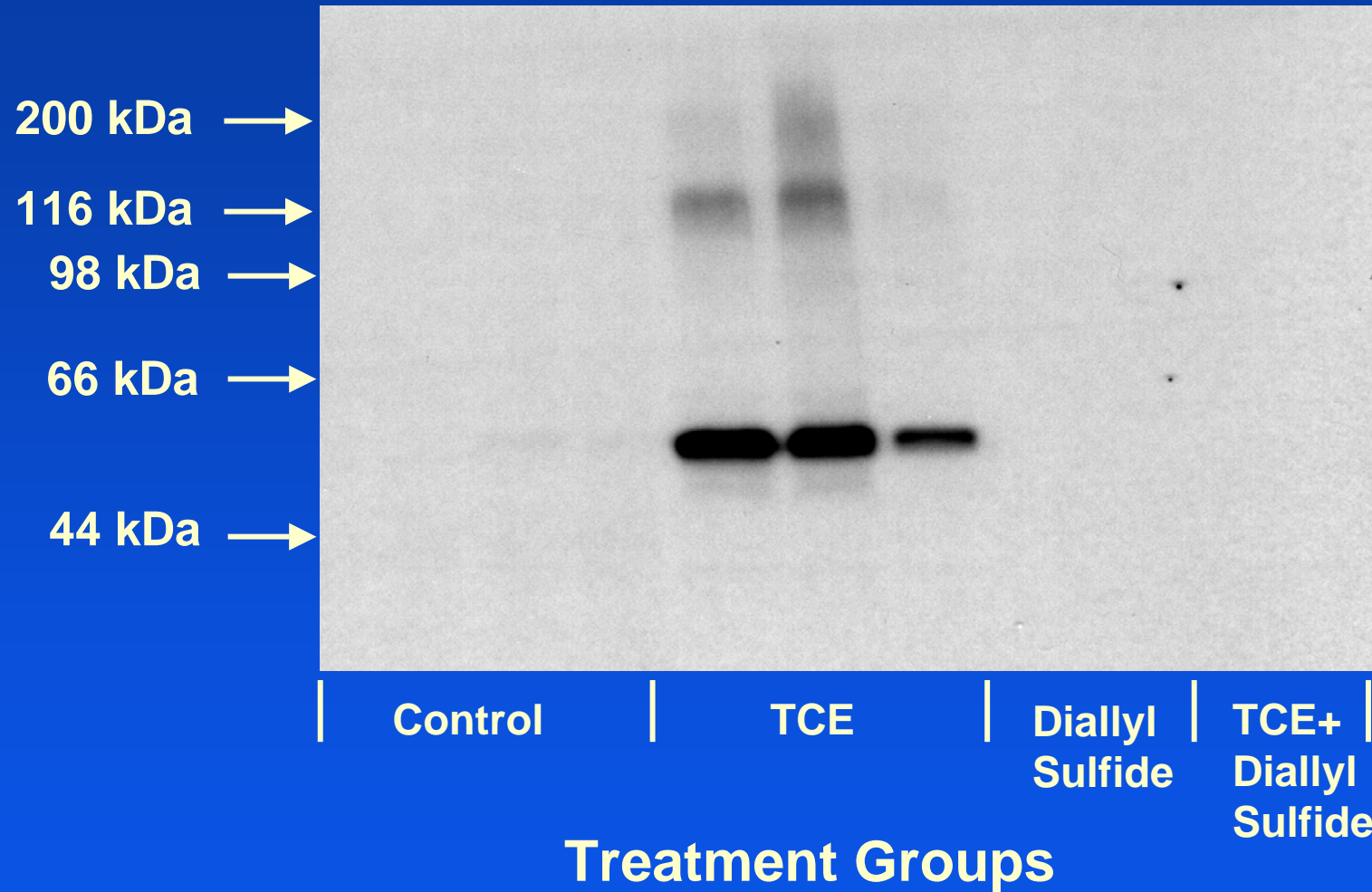




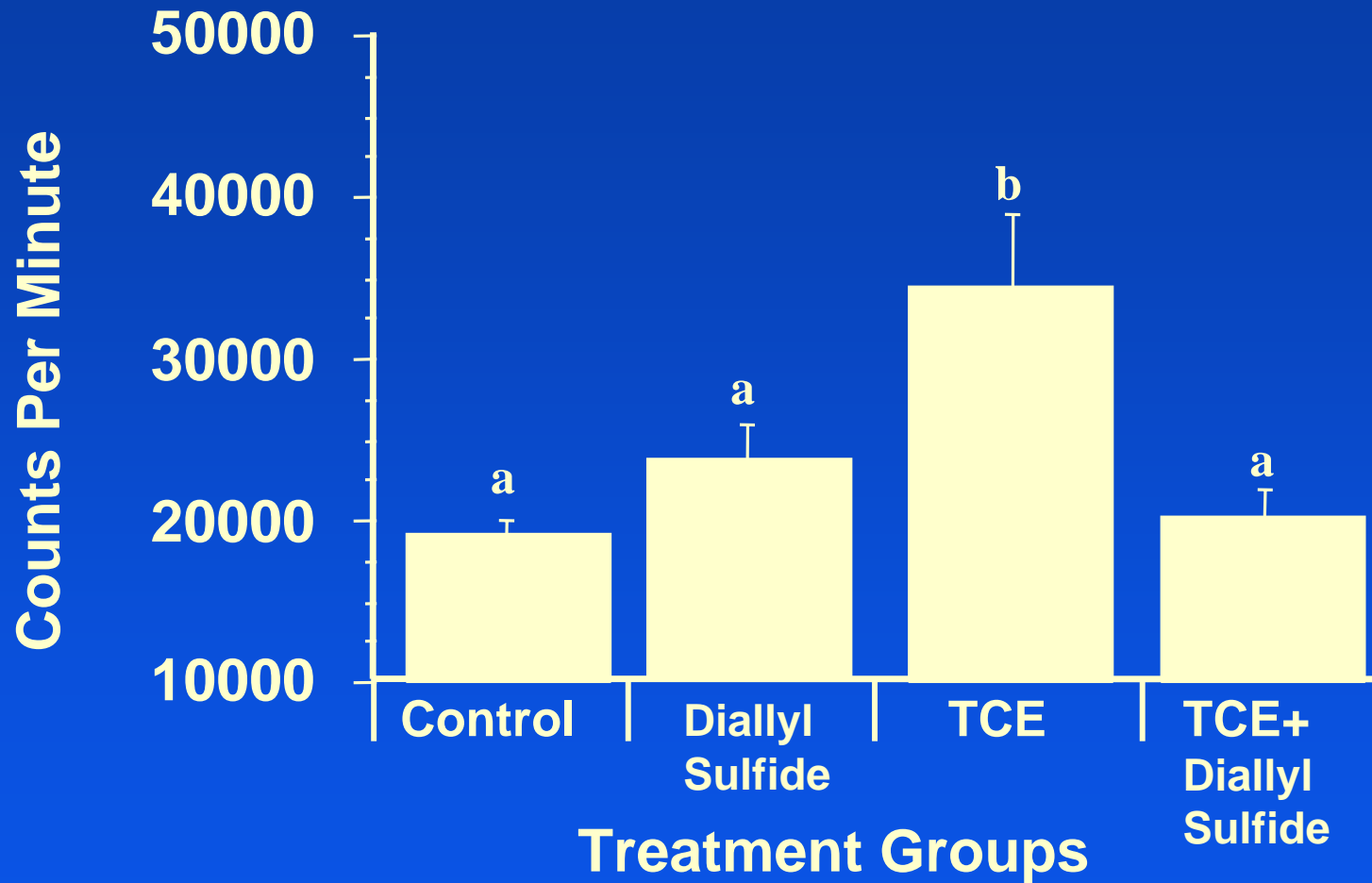
# Western Blot of CYP2E1 Following TCE Treatment of MRL+/+ Mice



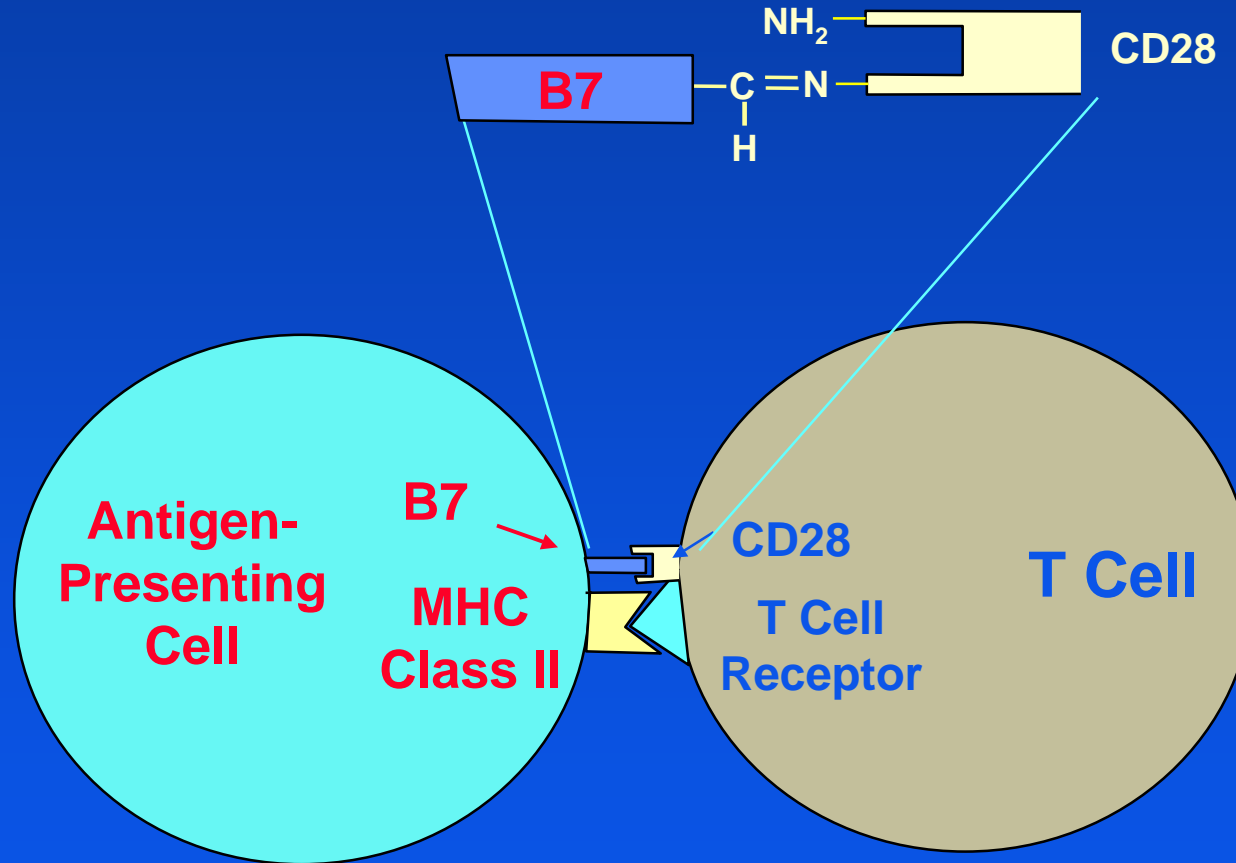
# Liver Trichloroethylene Adducts



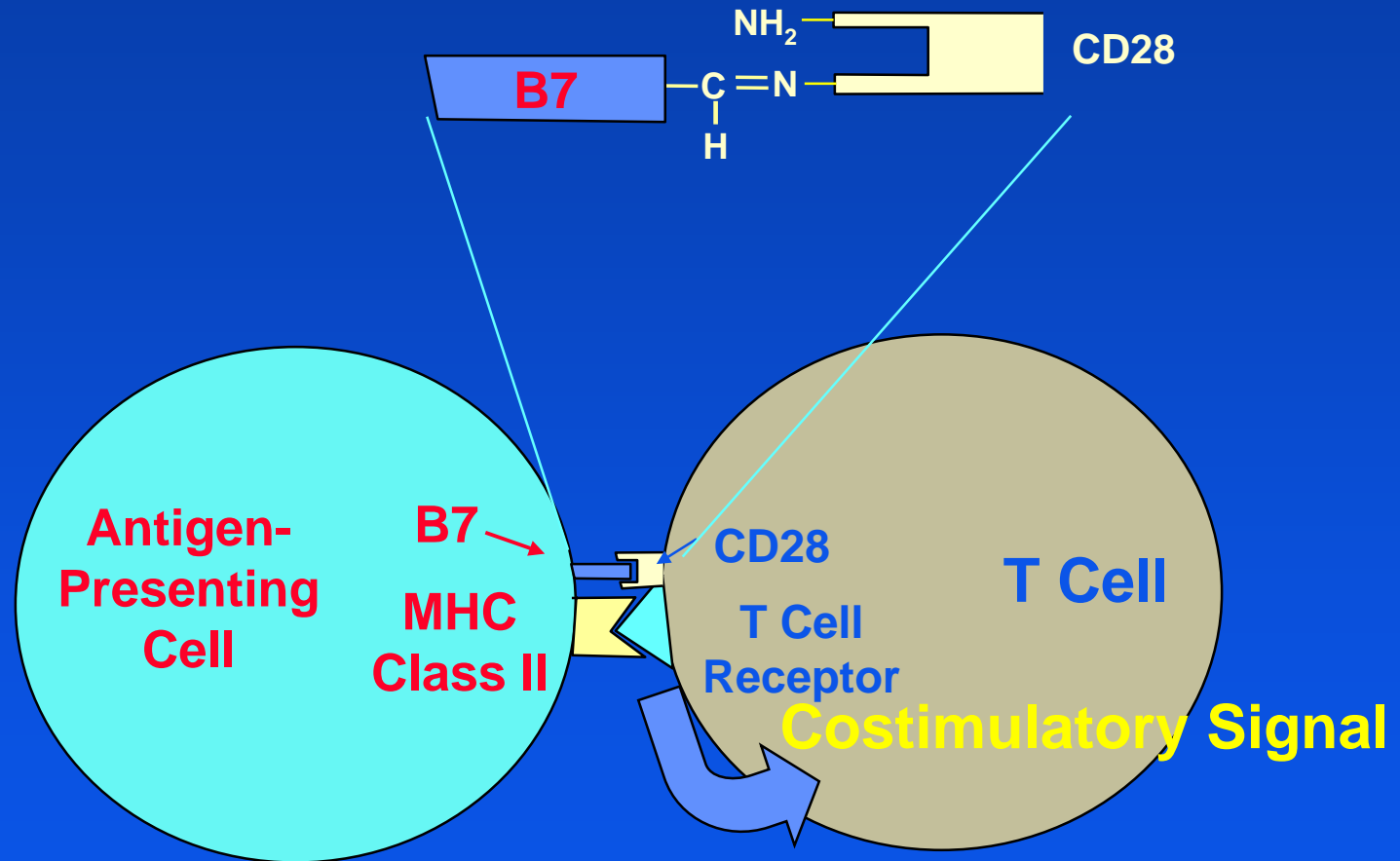
# CD4<sup>+</sup> T Cells Mitogenic Response to Con A



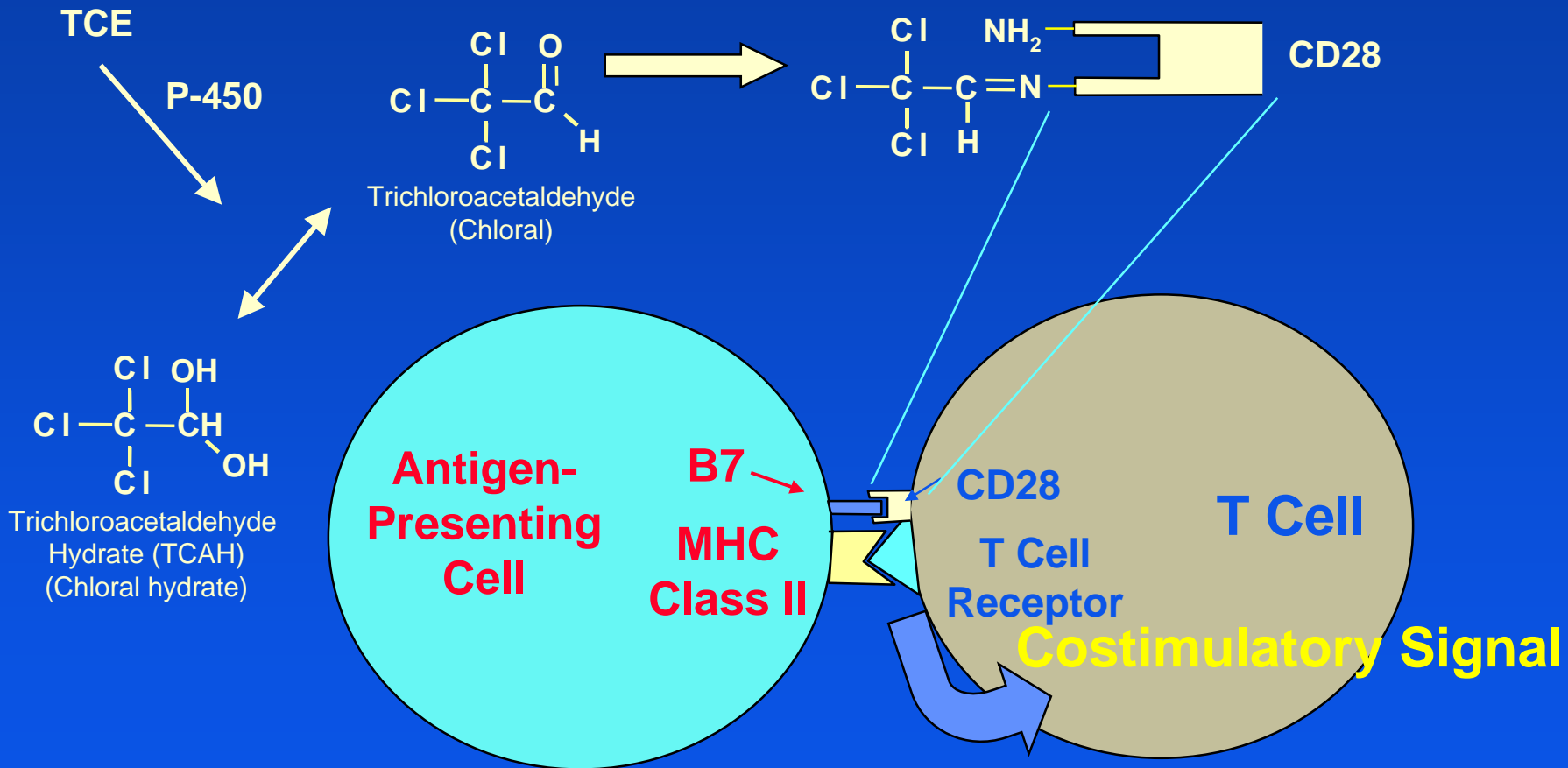
# T Cell Activation by Forming a Schiff-Base



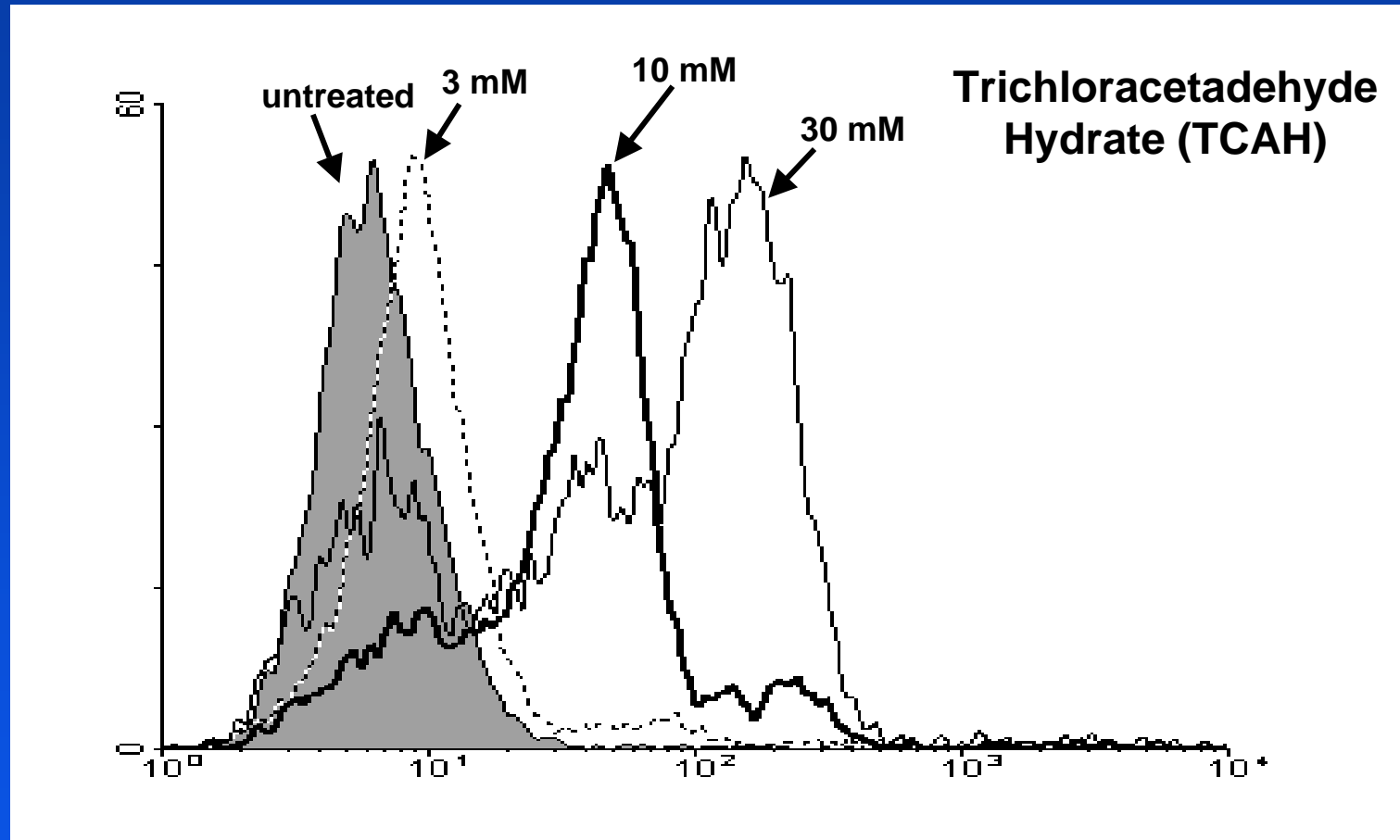
# T Cell Activation by Forming a Schiff-Base



# Trichloroethylene Metabolite Activation of T Cells by Forming a Schiff-Base

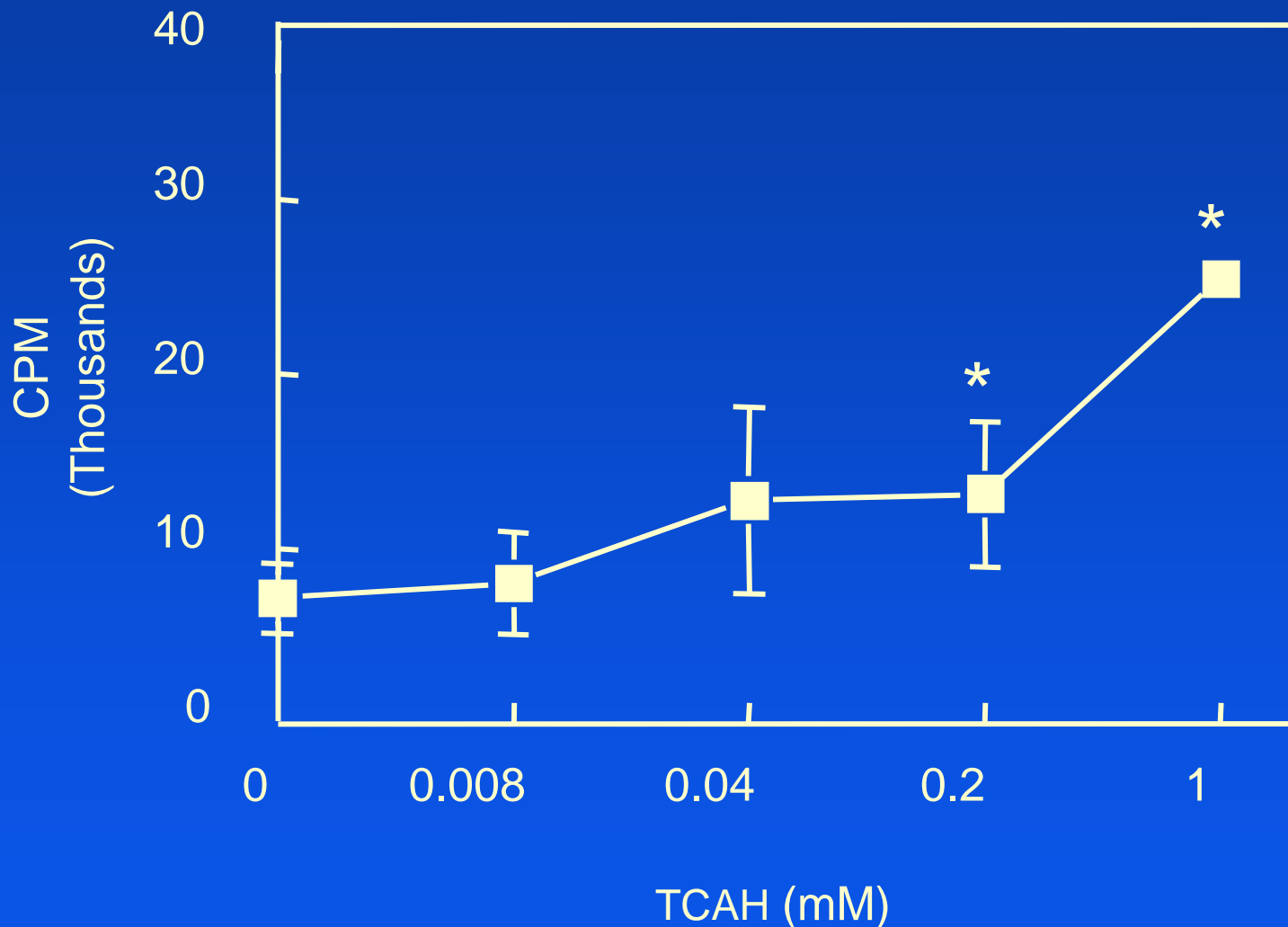


# Trichloroacetaldehyde Binding to Th1 Cell Surface Protein



Fluorescence Intensity

# Trichloroacetaldehyde Hydrate (TCAH) Costimulates T-Cell Proliferation *in vitro* (0.01 $\mu\text{g/ml}$ anti-CD3)



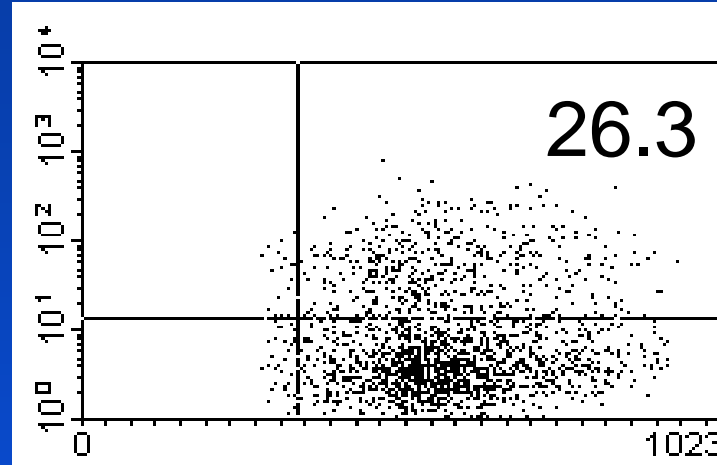
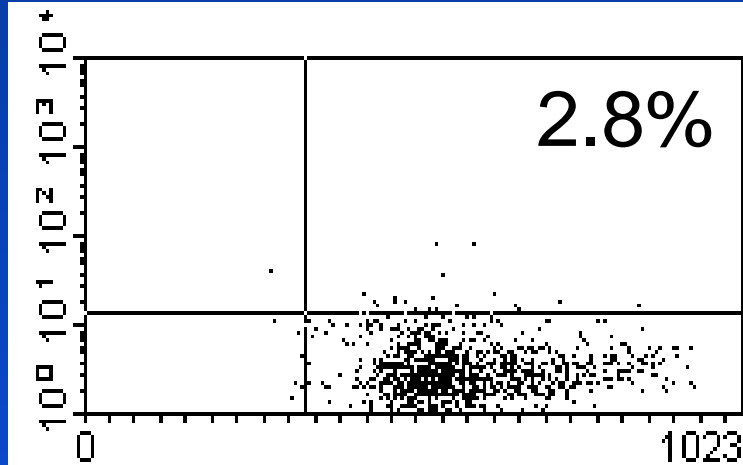


# TCAH Stimulates Th1 Cells Activation

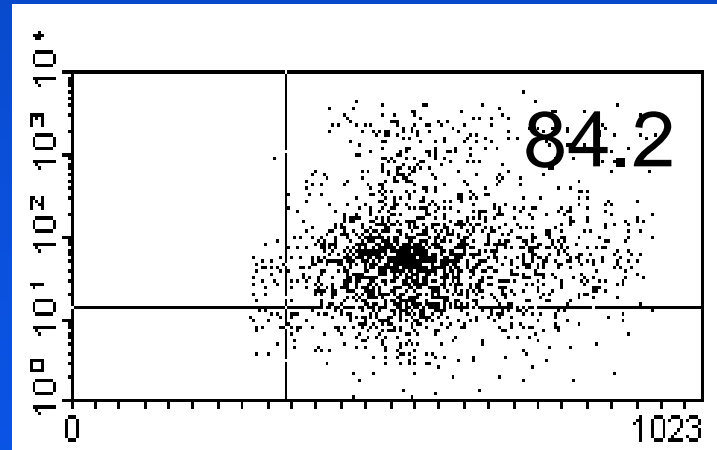
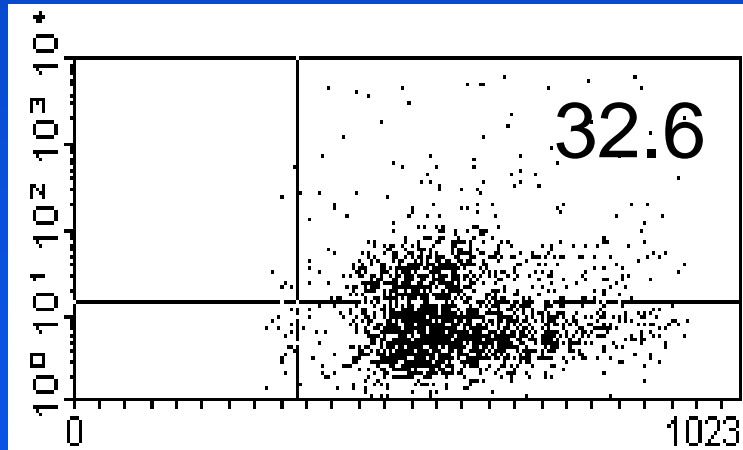
No Anti-CD3

Anti-CD3

CD28



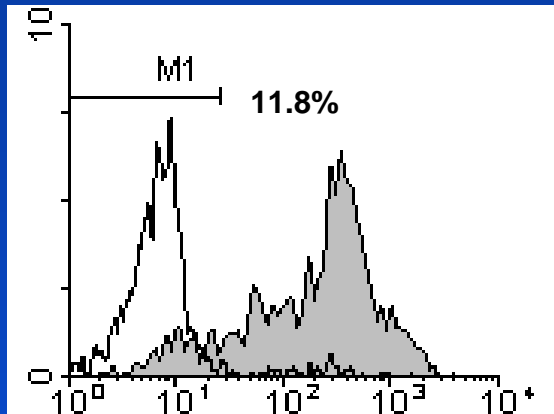
No  
TCAH



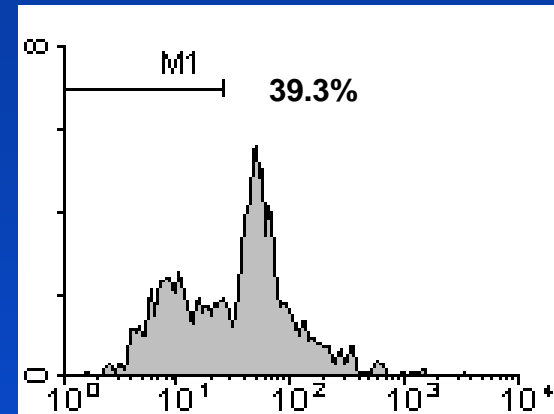
TCAH  
(1 mM)

# Trichloroacetaldehyde Hydrate (TCAH) Stimulates Activation (CD62L<sup>lo</sup>) in CD4<sup>+</sup> T Cells

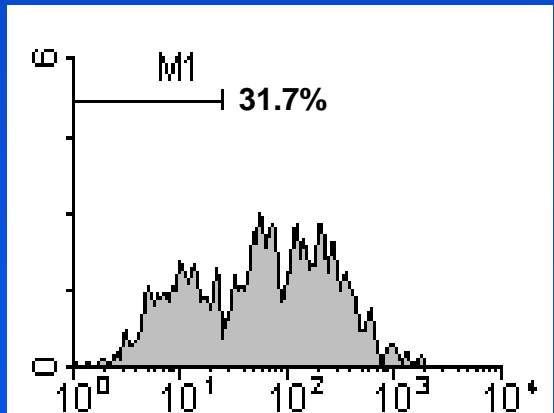
0 mM TCAH



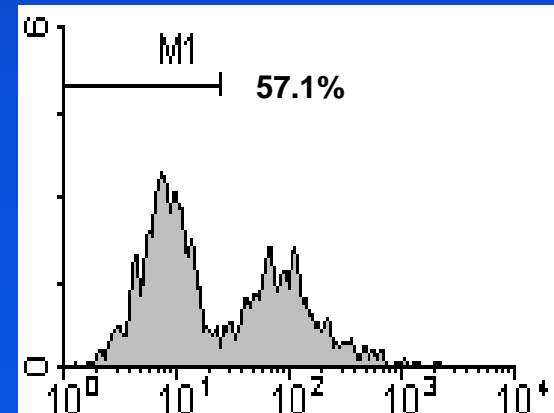
0.2 mM TCAH



0.04 mM TCAH



1 mM TCAH



Relative number CD62L<sup>lo</sup>

Fluorescence Intensity

Fluorescence Intensity

# Experimental Design

Autoimmune-Prone  
MRL+/+ Mice



Trichloroacetaldehyde  
Hydrate



Serum  
Spleen & lymph nodes  
Liver & lungs

Toxicity  
Autoimmune markers

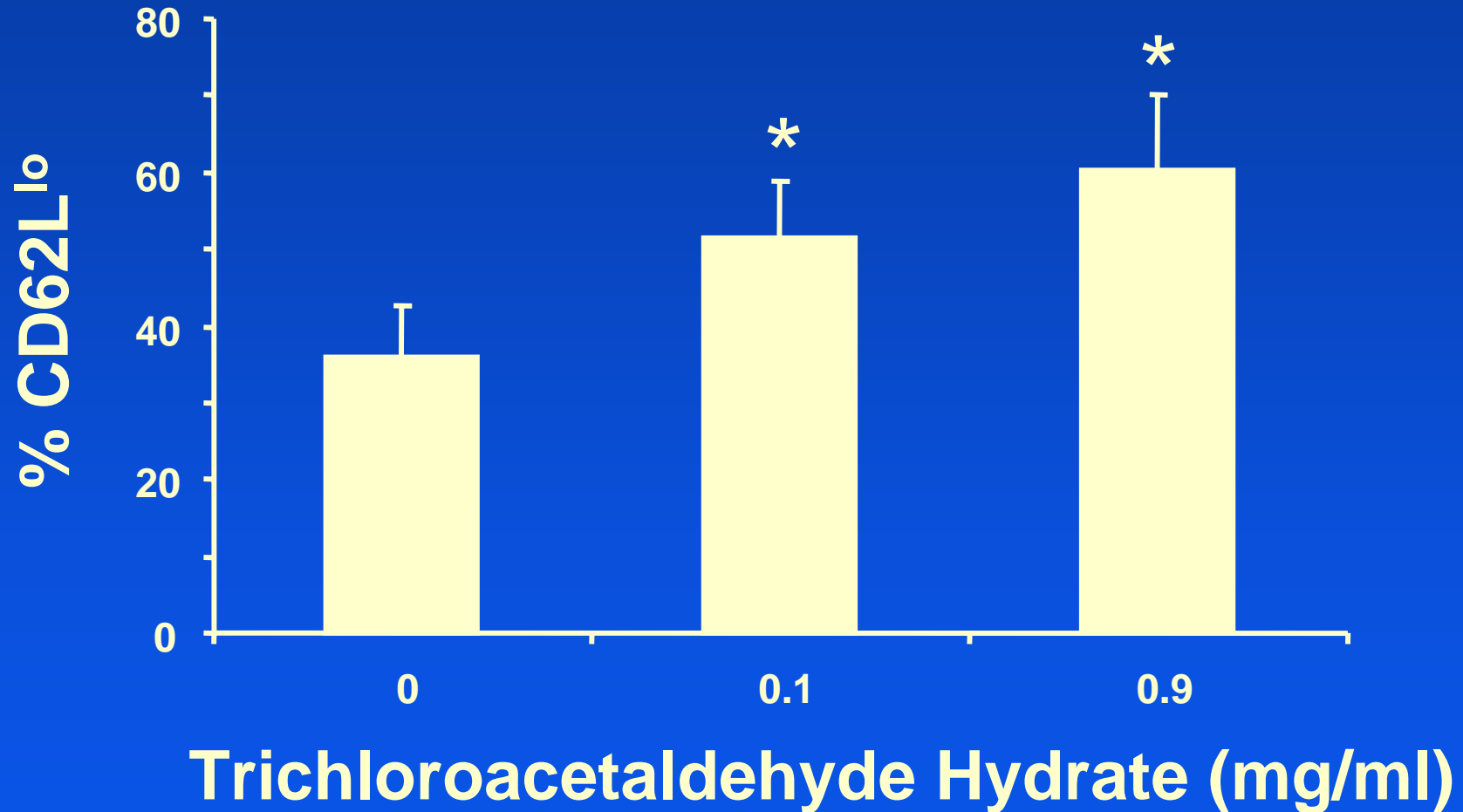
T-cell Activation  
Cytokines (IFN $\gamma$  & IL-4)

Metabolic Activation  
Markers of Oxidative stress



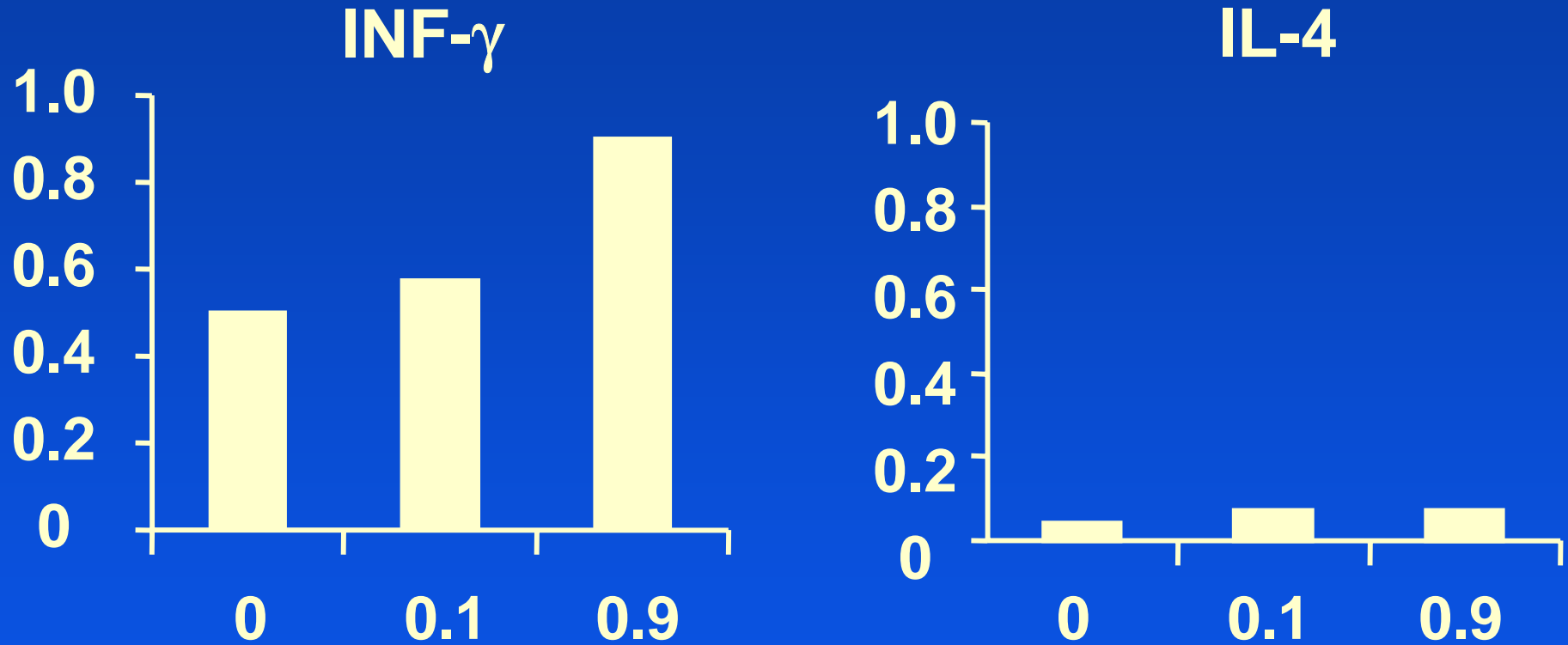
# Expression of CD62L<sup>lo</sup> on T cells

(MRL+/+ mice 4 weeks *in vivo* treatment)



# T cells Cytokine Secretion

(MRL+/+ mice 4 weeks *in vivo* treatment)



Trichloroacetaldehyde Hydrate mg/ml

# Acknowledgements

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Joseph Griffin, Ph.D.

## Support

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USDOE

NIOSH

# References

Gilbert, K.M, Griffin, J.M, and Pumford, N.R. Trichloroethylene activates CD4+ T cells: potential role in an autoimmune response. *Drug. Metab. Rev.*,31(4), 901-916, 1999.

Griffin, J.M., Blossom, S.J., Jackson, S.K., Gilbert, K.M., and Pumford, N.R. Trichloroethylene accelerates an autoimmune response in association with Th1 T cell activation in MRL/++ mice. *Immunopharmacology* 46(2): 123-37, 2000

Griffin, J.M., Gilbert, K.M., and Pumford, N.R. Inhibition of CYP2E1 reverses CD4+ T cell alterations in trichloroethylene-treated MRL+/+ mice. *Toxicol. Sci.*, 54: 384-389, 2000.

Griffin, J.M., Gilbert, K.M., Lamps, L.W., and Pumford, N.R. CD4+ T cell activation and induction of autoimmune hepatitis following trichloroethylene treatment in MRL+/+ mice. *Toxicol. Sci.*, 57: 345-352, 2000.

Gilbert, K. M., Whitlow, A. B., and Pumford, N.R. Environmental contaminant and disinfection by-product stimulates T cells *in vitro*. *International Immunopharmacology* 4(1):25-36, 2004.