

TCE and T Cell Activation



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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

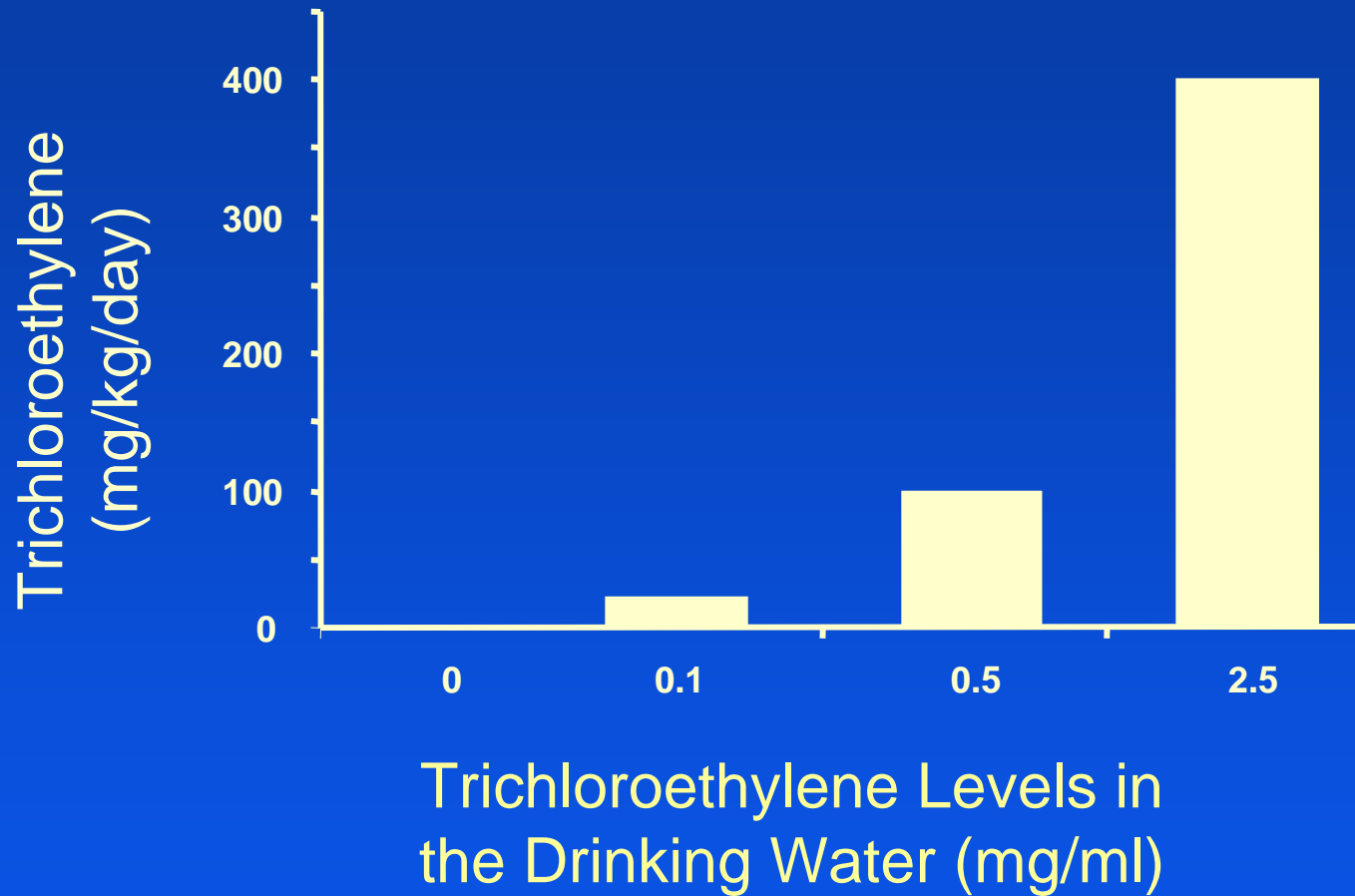
Toxicity
Autoimmune markers

T-cell Activation
Cytokines (IFN γ & IL-4)

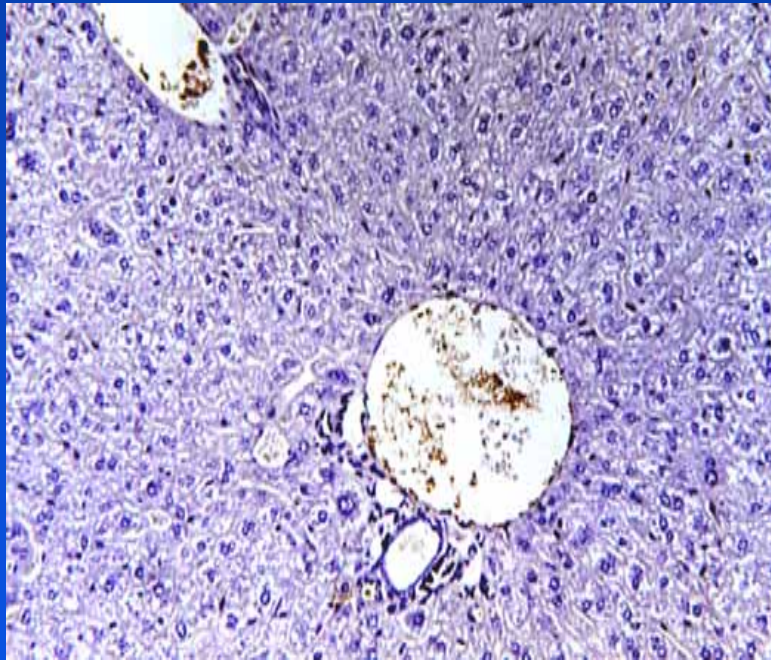
Metabolic Activation
Markers of Oxidative stress



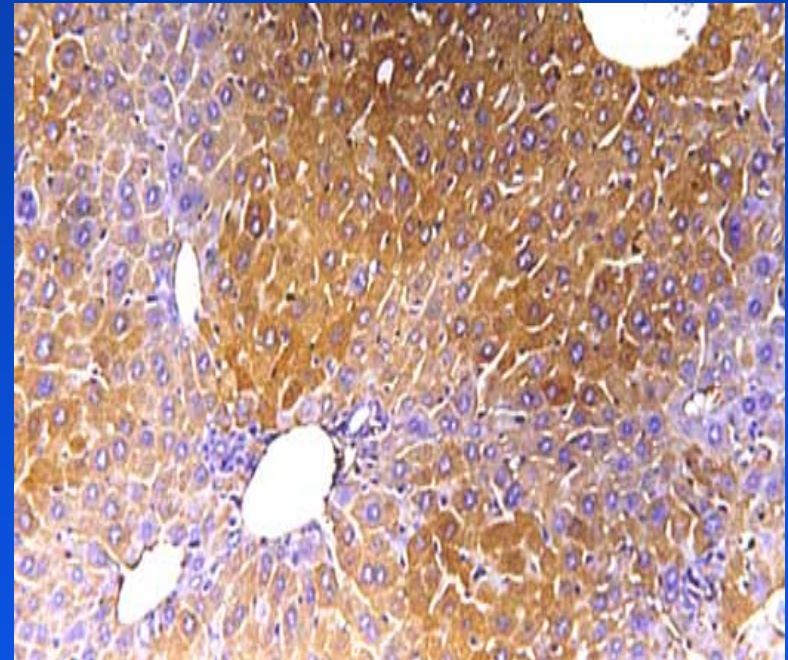
Low-Dose Chronic Study



Immunohistochemistry for Trichloroethylene-Protein Adducts



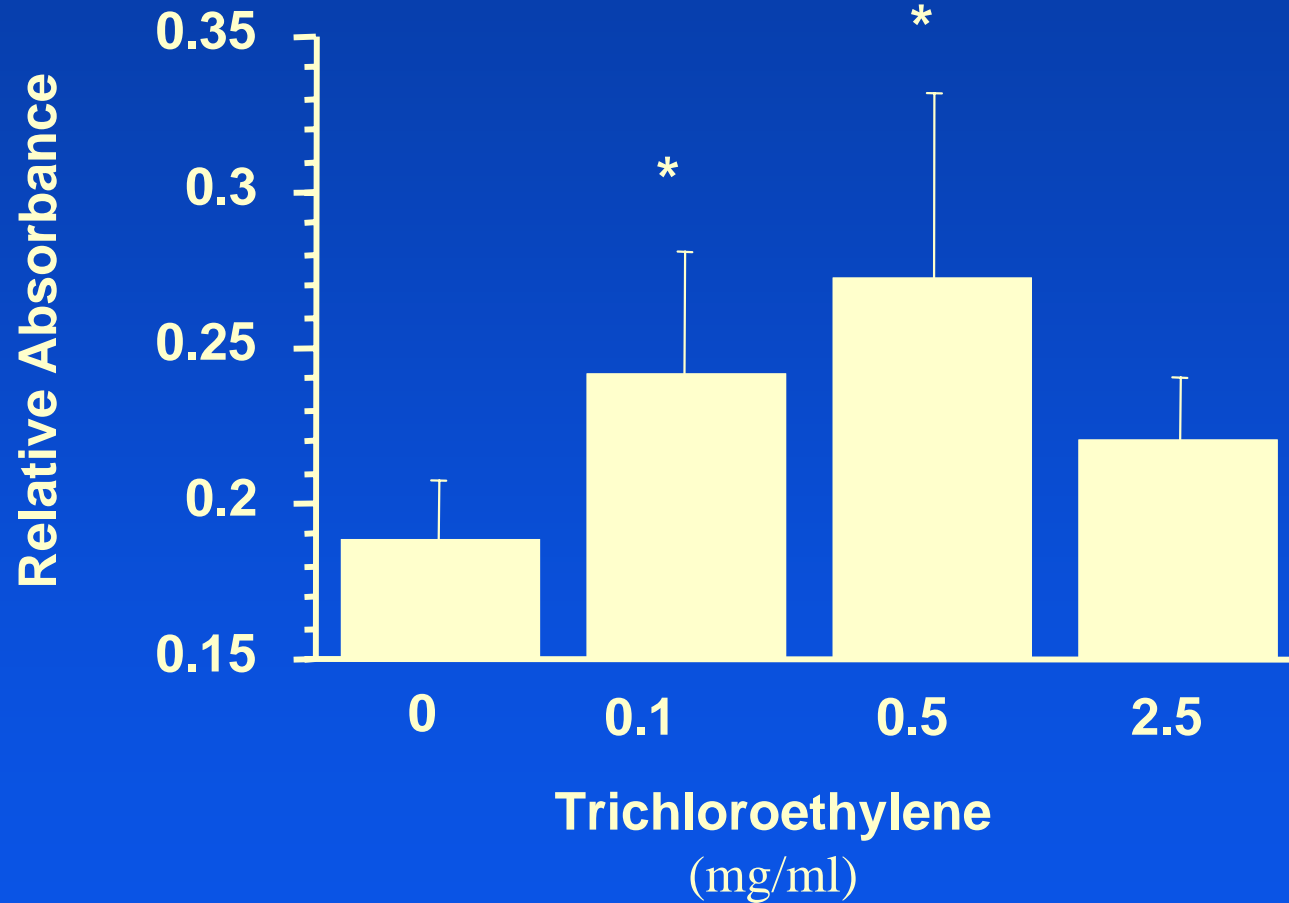
Control





Trichloroethylene

Antinuclear Antibodies

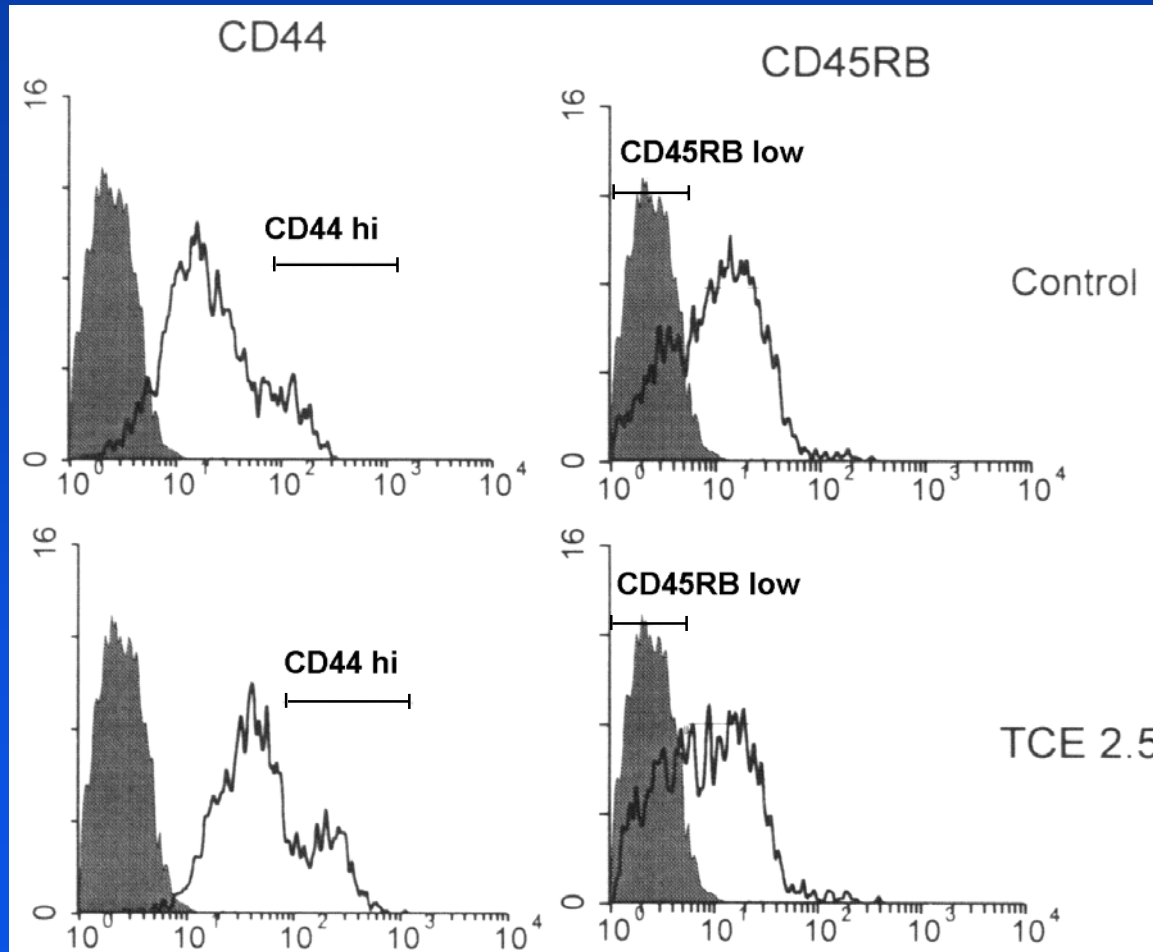
4 Weeks



Activation of CD4+ T Cells

-  CD44 expression on CD4⁺ 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

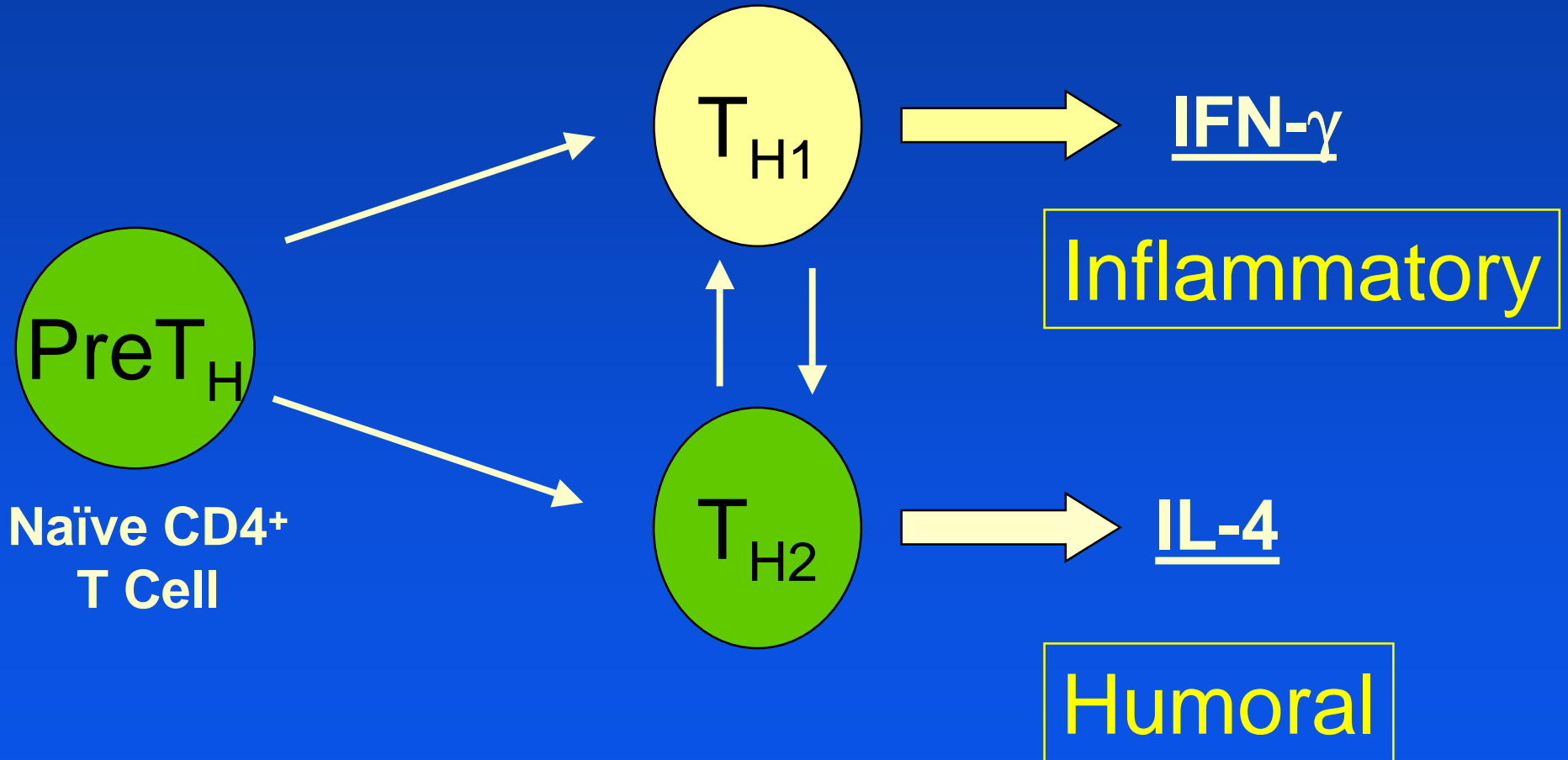
Expression of CD44 and CD45RB from Mice Treated for 4 Weeks with Trichloroethylene



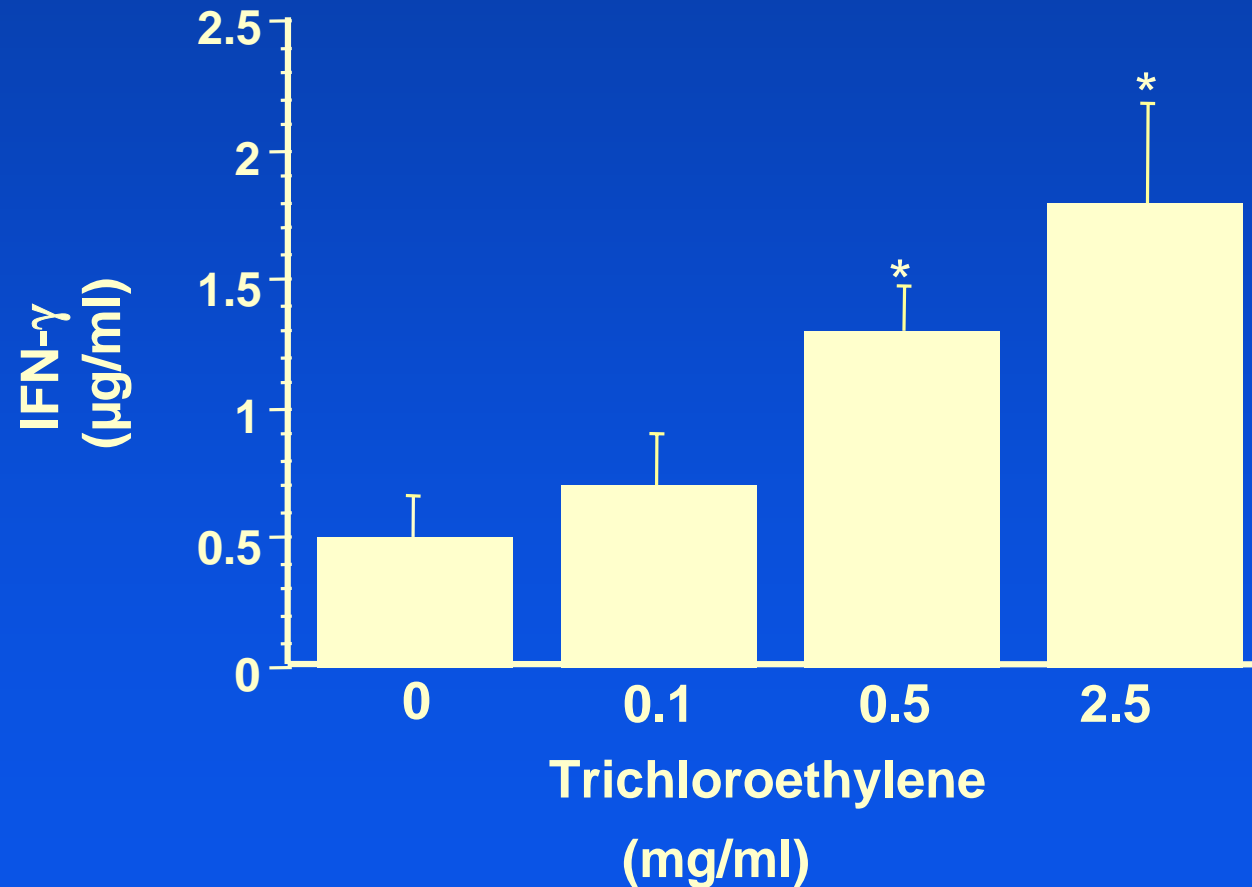
CD4⁺ T Cell Activation in Spleens following treatment with Trichloroethylene

	CD44^{hi} % Total Cells	CD45RB^{low} % Total Cells
Control	39	58
0.1 TCE	39	59
0.5 TCE	44	64
2.5 TCE	53	69

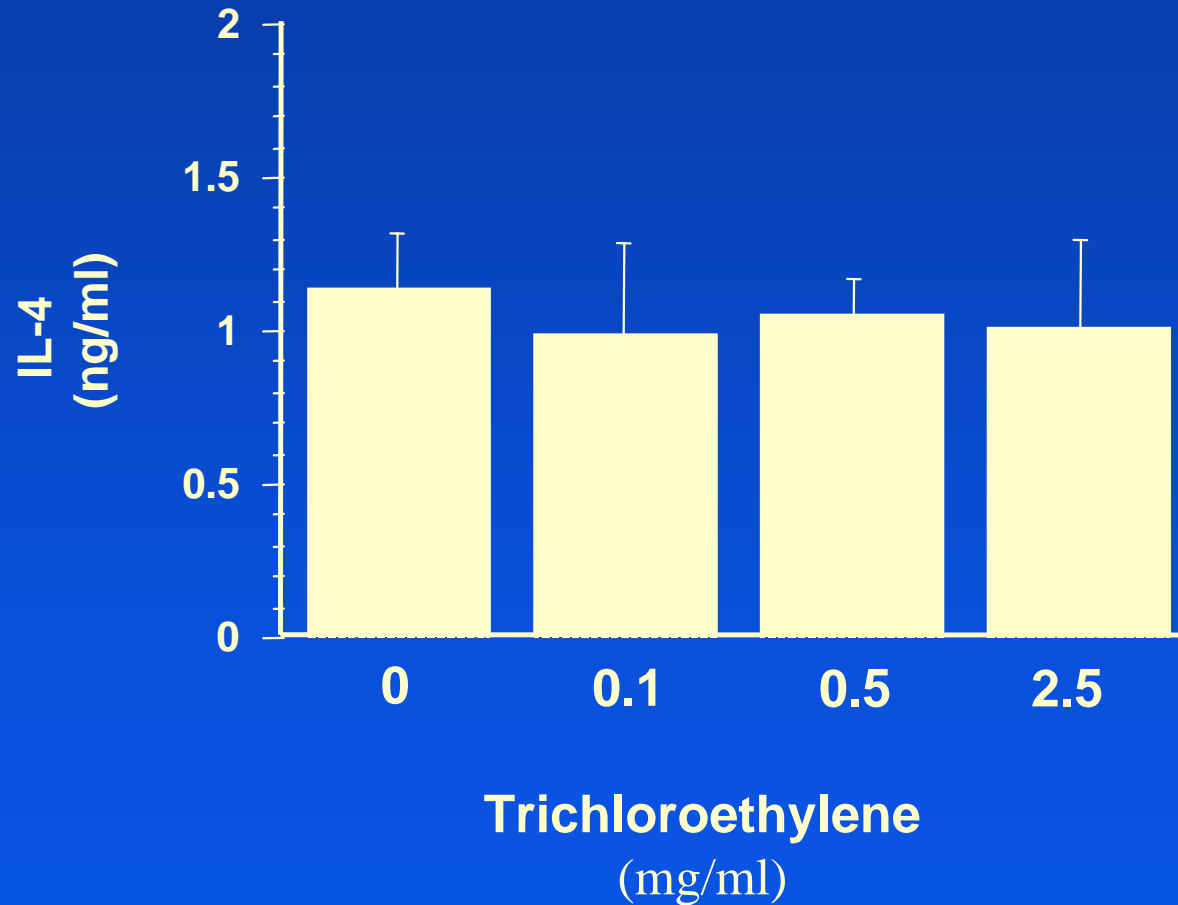
CD4⁺ T Cell Maturation



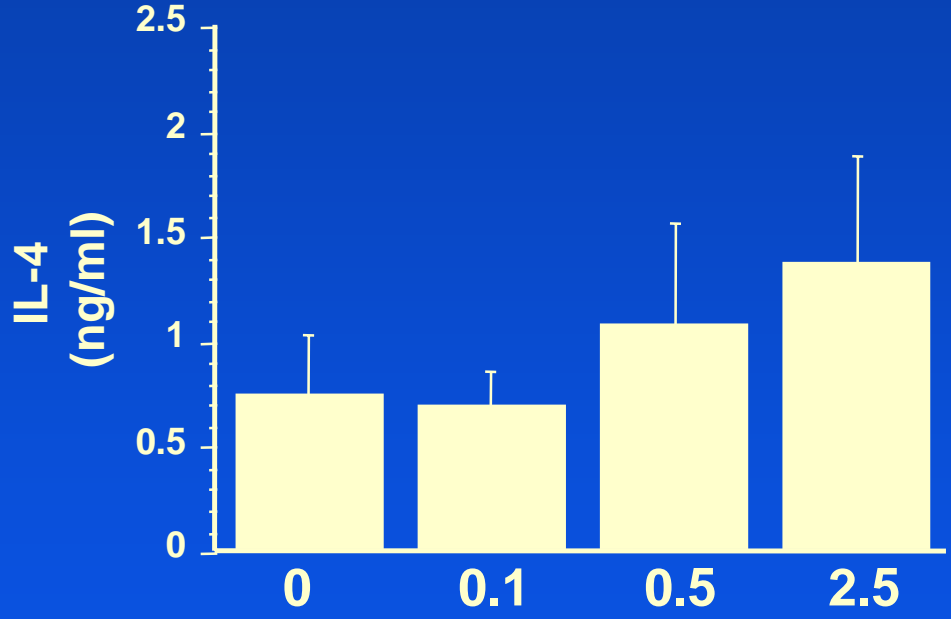
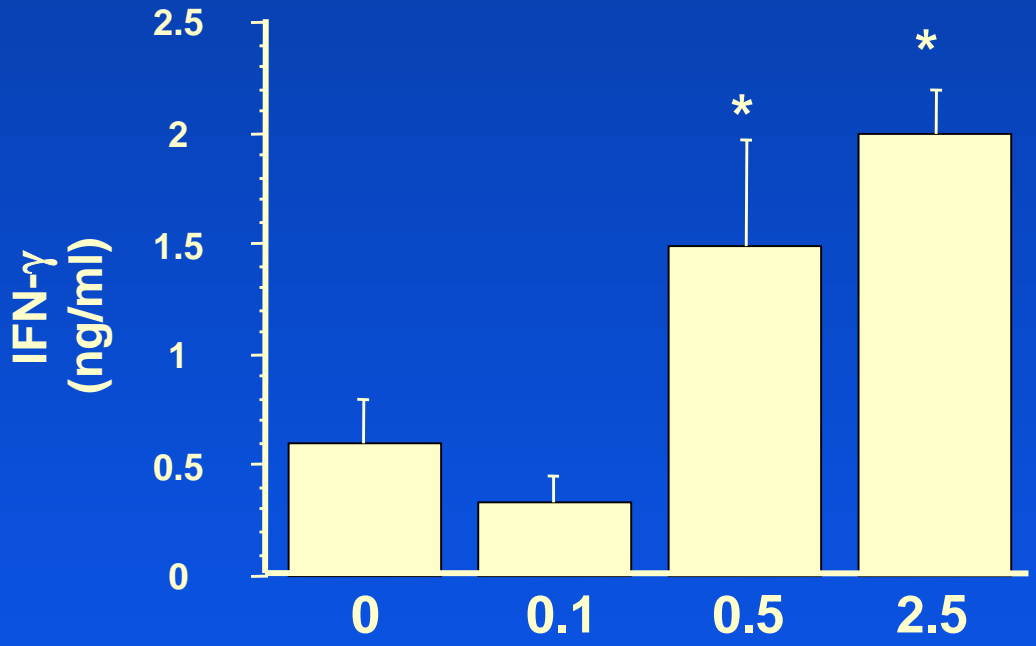
Interferon- γ Levels in MRL^{+/+} Mice (4 weeks)



IL-4 Levels in MRL^{+/+} Mice (4 weeks)



Cytokine Levels (32 weeks)

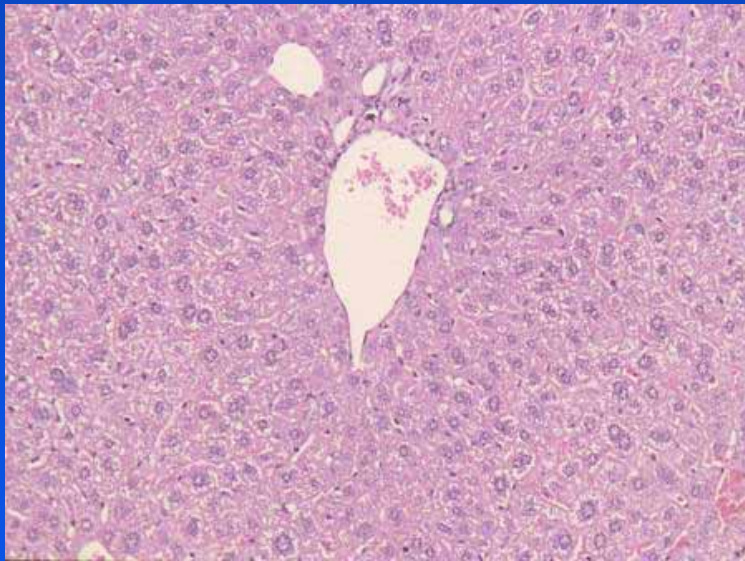
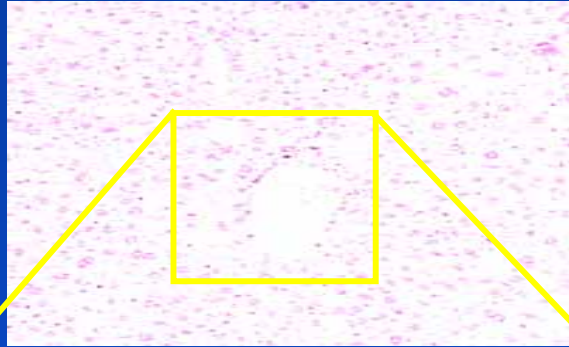


Trichloroethylene
(mg/ml)

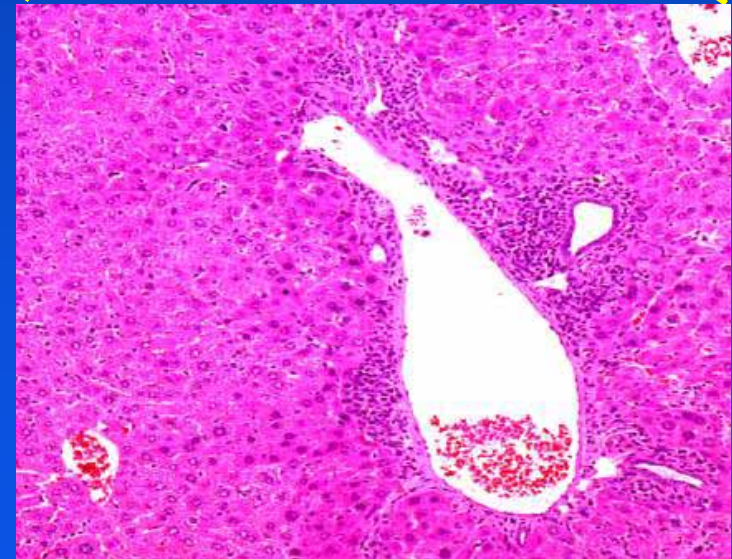
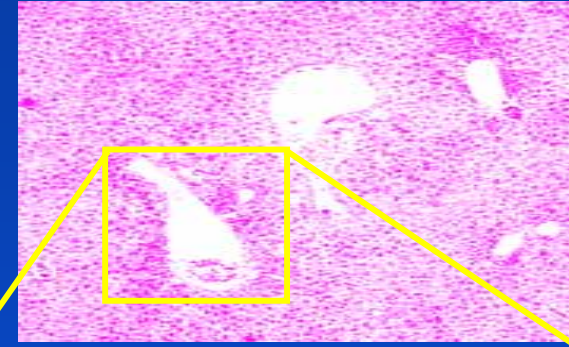
Toxicity Testing

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

Liver Histology

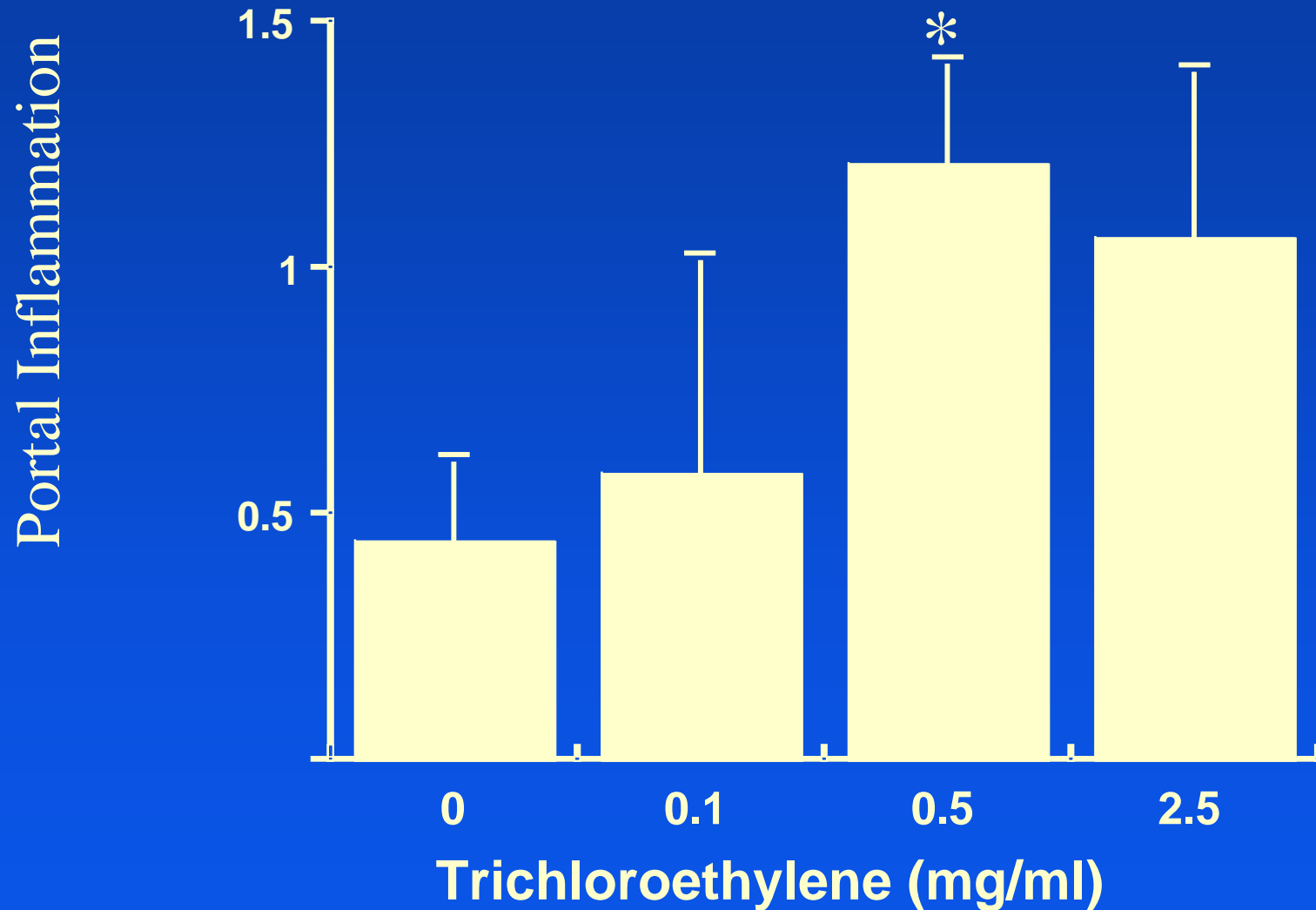


Control

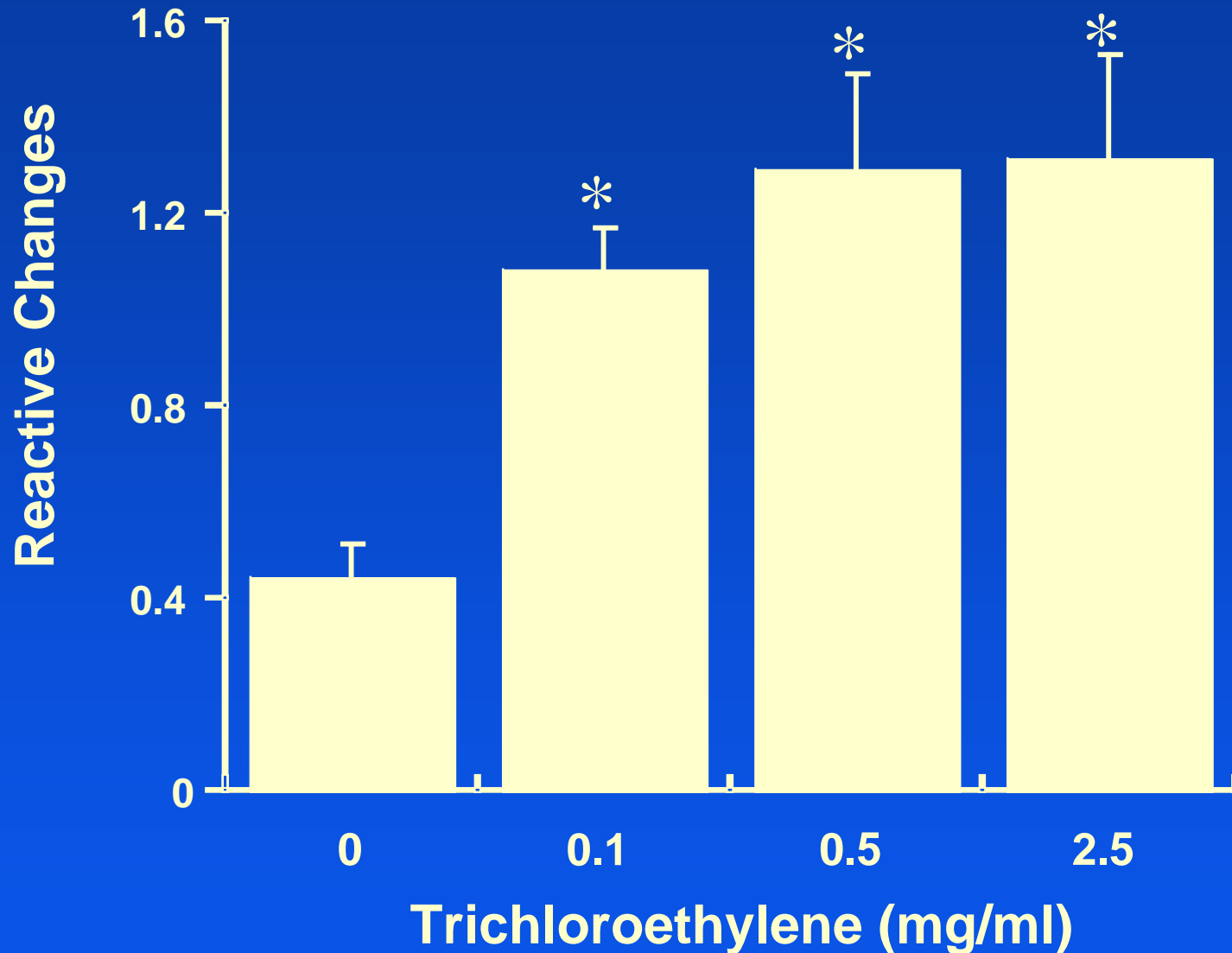


TCE

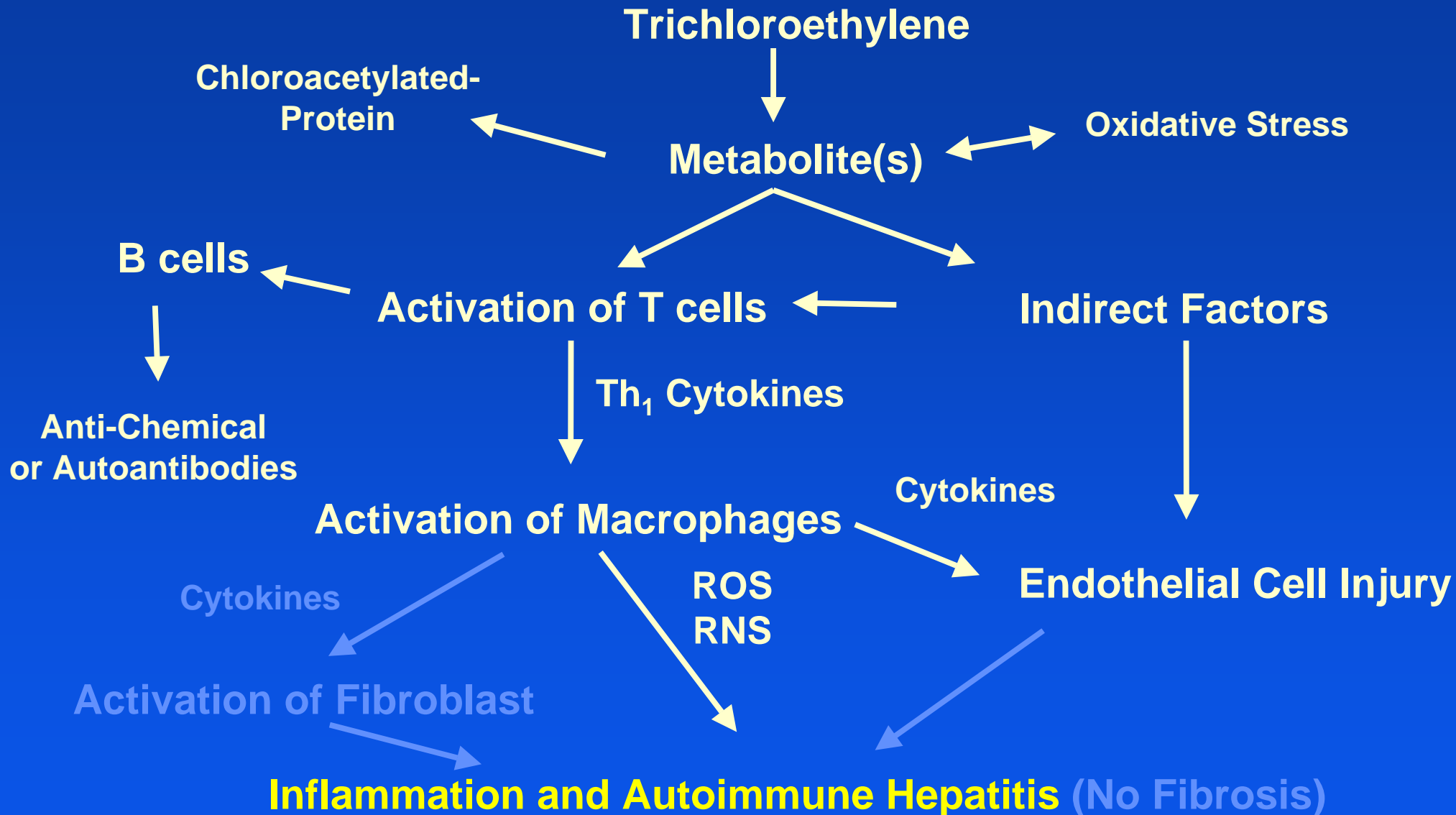
Liver Histopathological Score



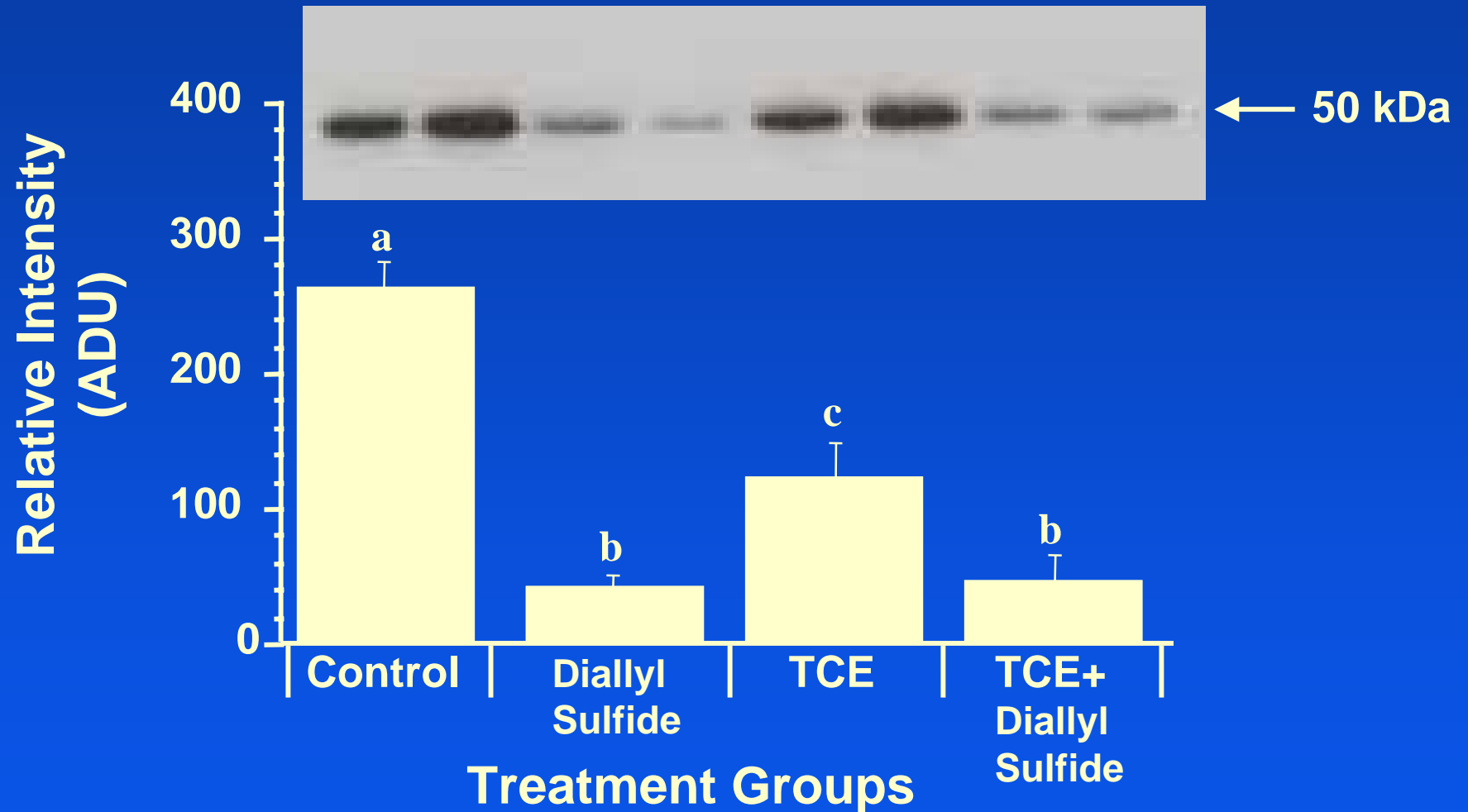
Liver Histopathological Score



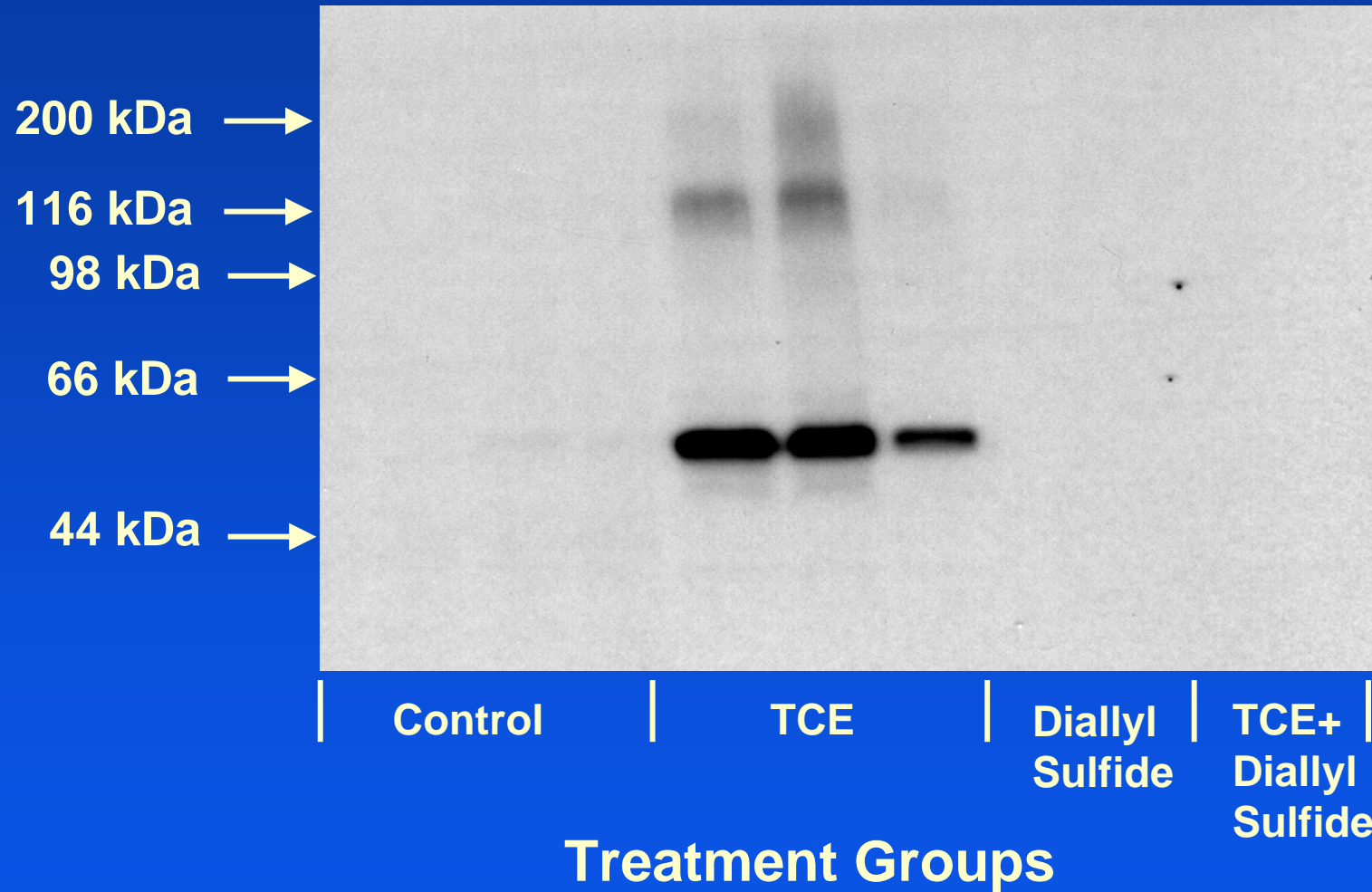
Mechanism of Trichloroethylene-Induced Autoimmunity



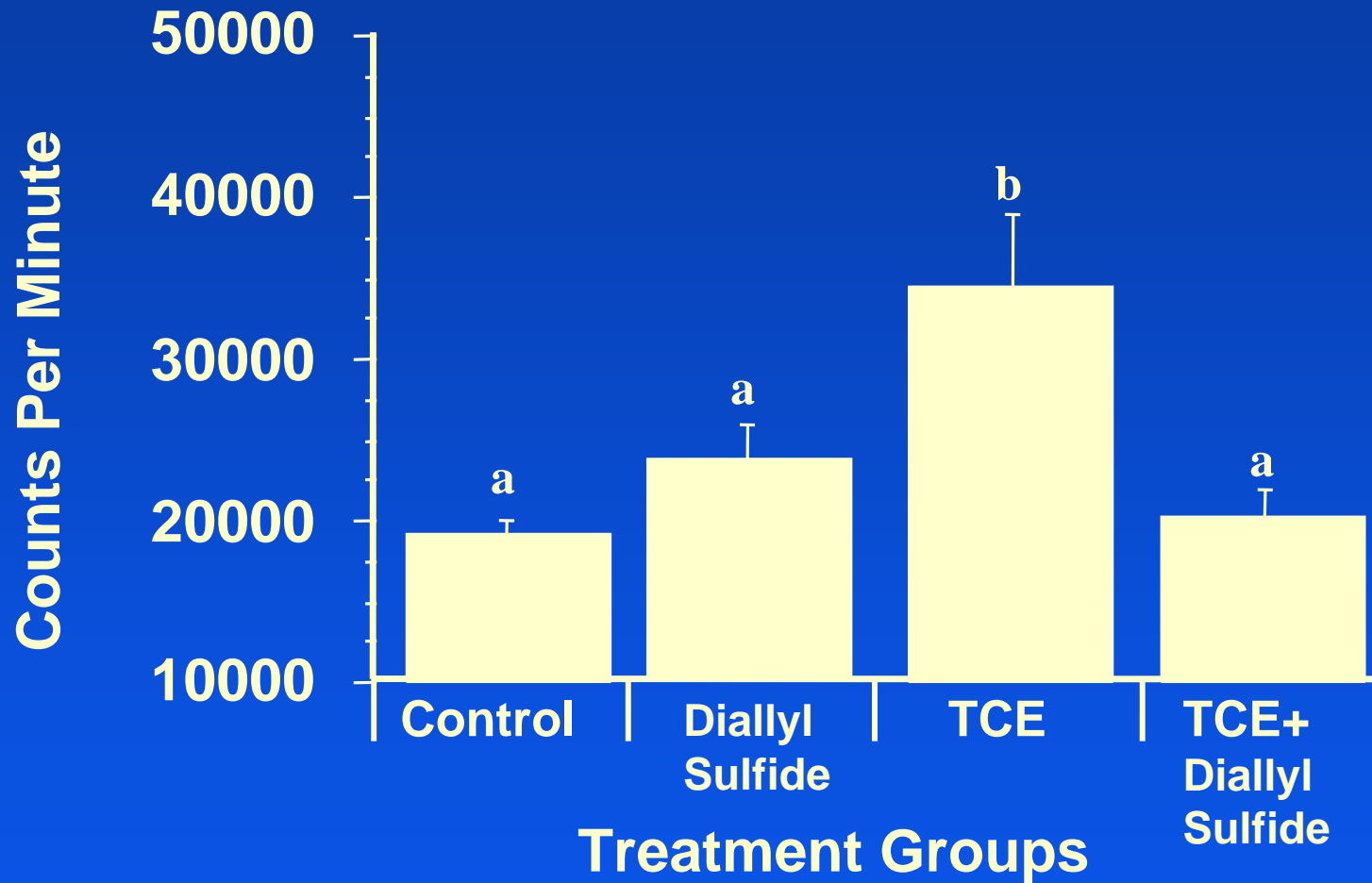
Western Blot of CYP2E1 Following TCE Treatment of MRL+/+ Mice



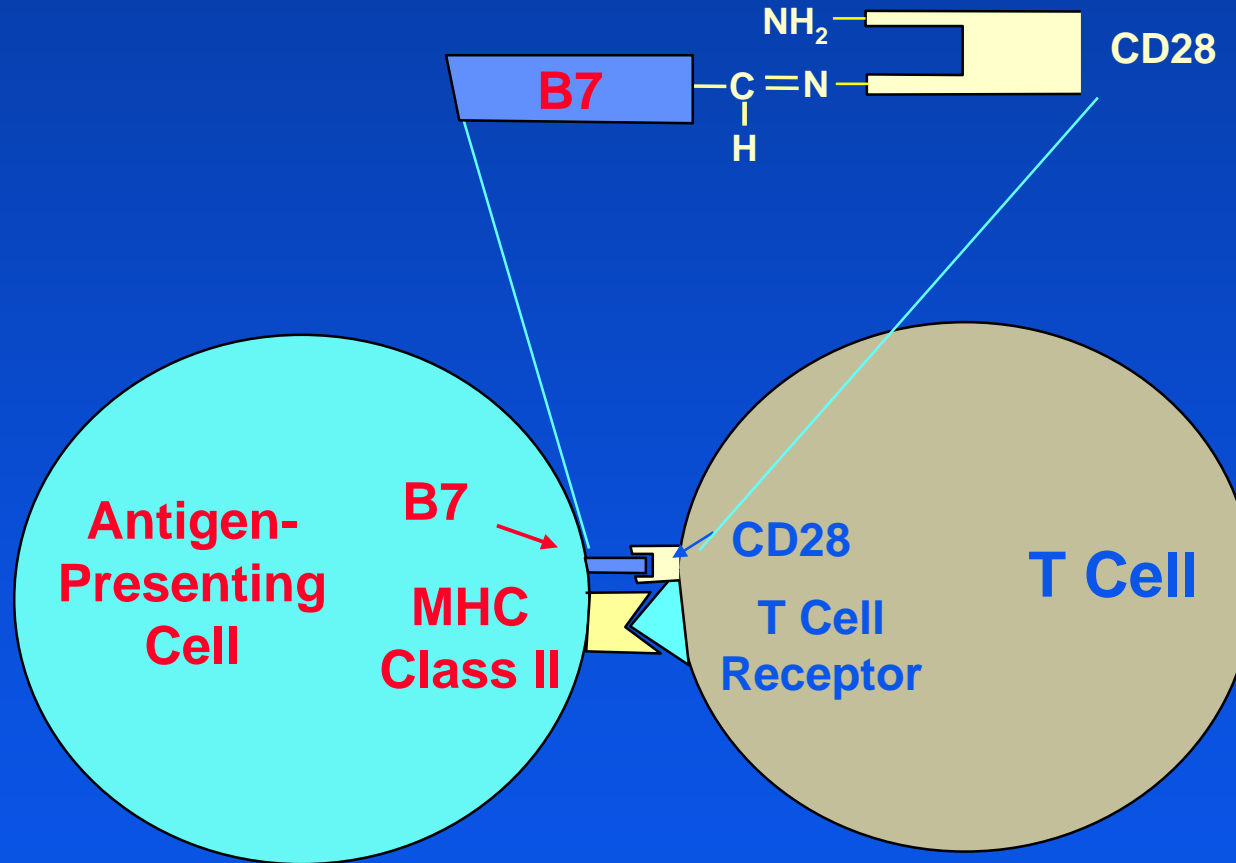
Liver Trichloroethylene Adducts



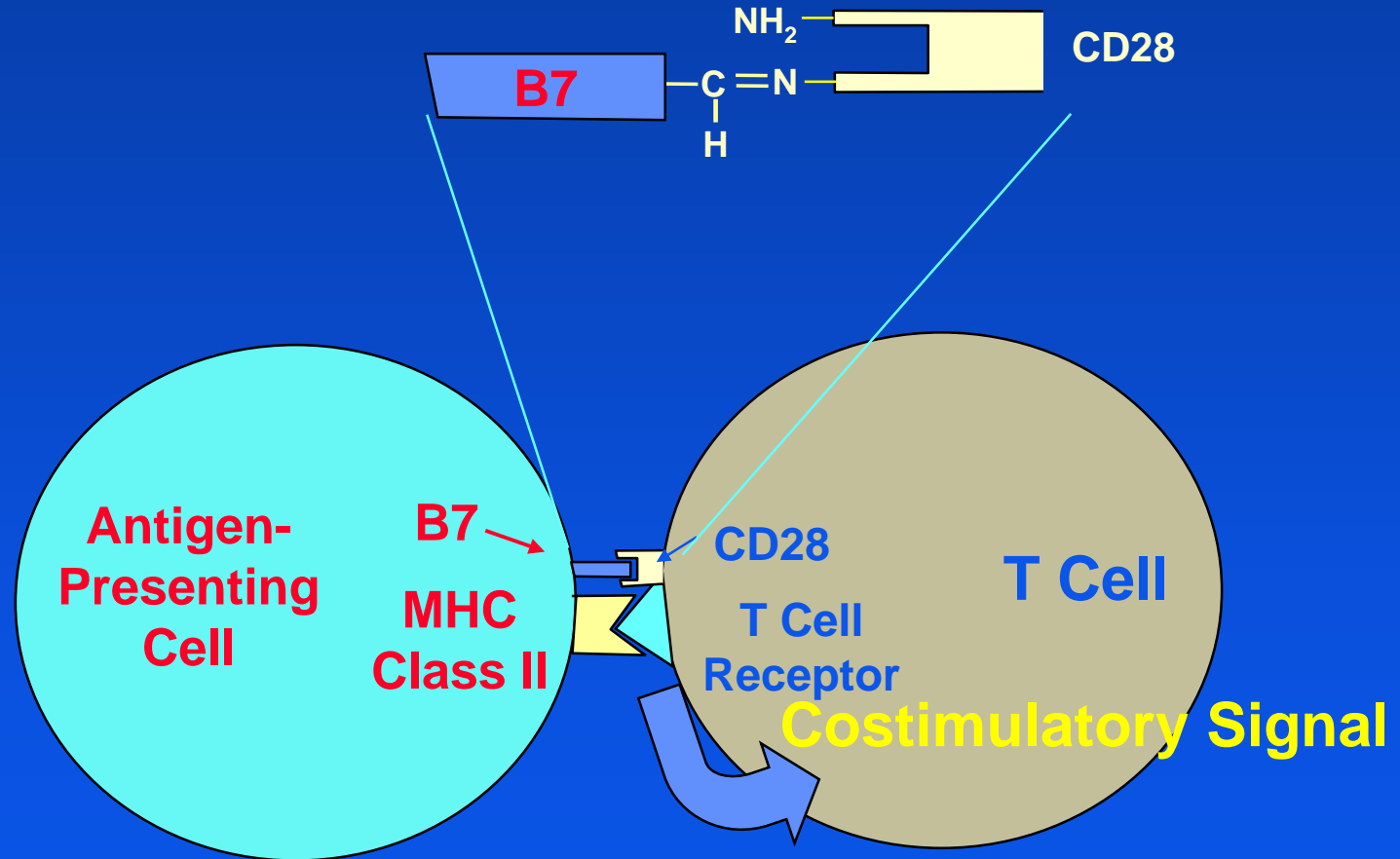
CD4⁺ T Cells Mitogenic Response to Con A



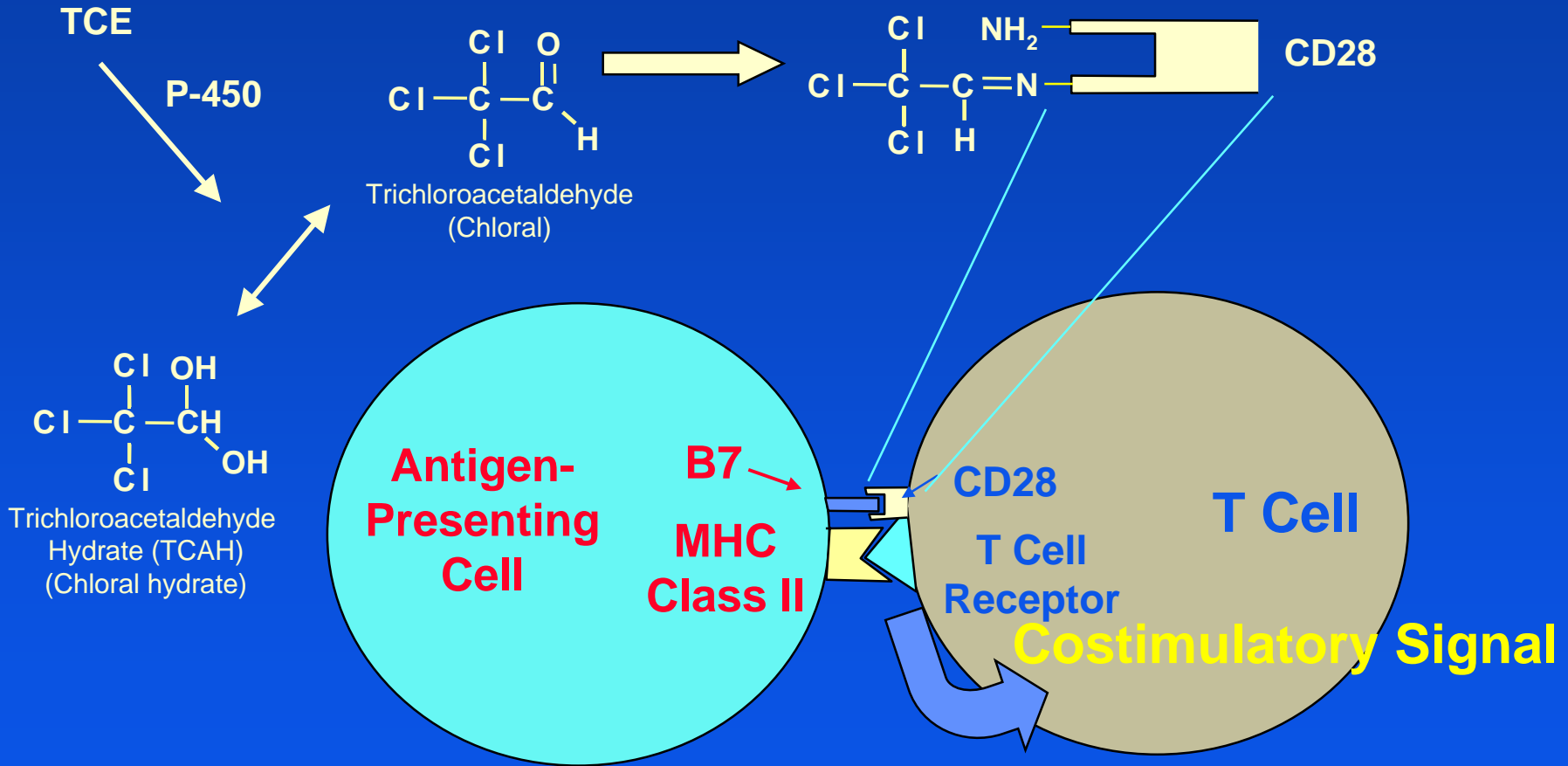
T Cell Activation by Forming a Schiff-Base



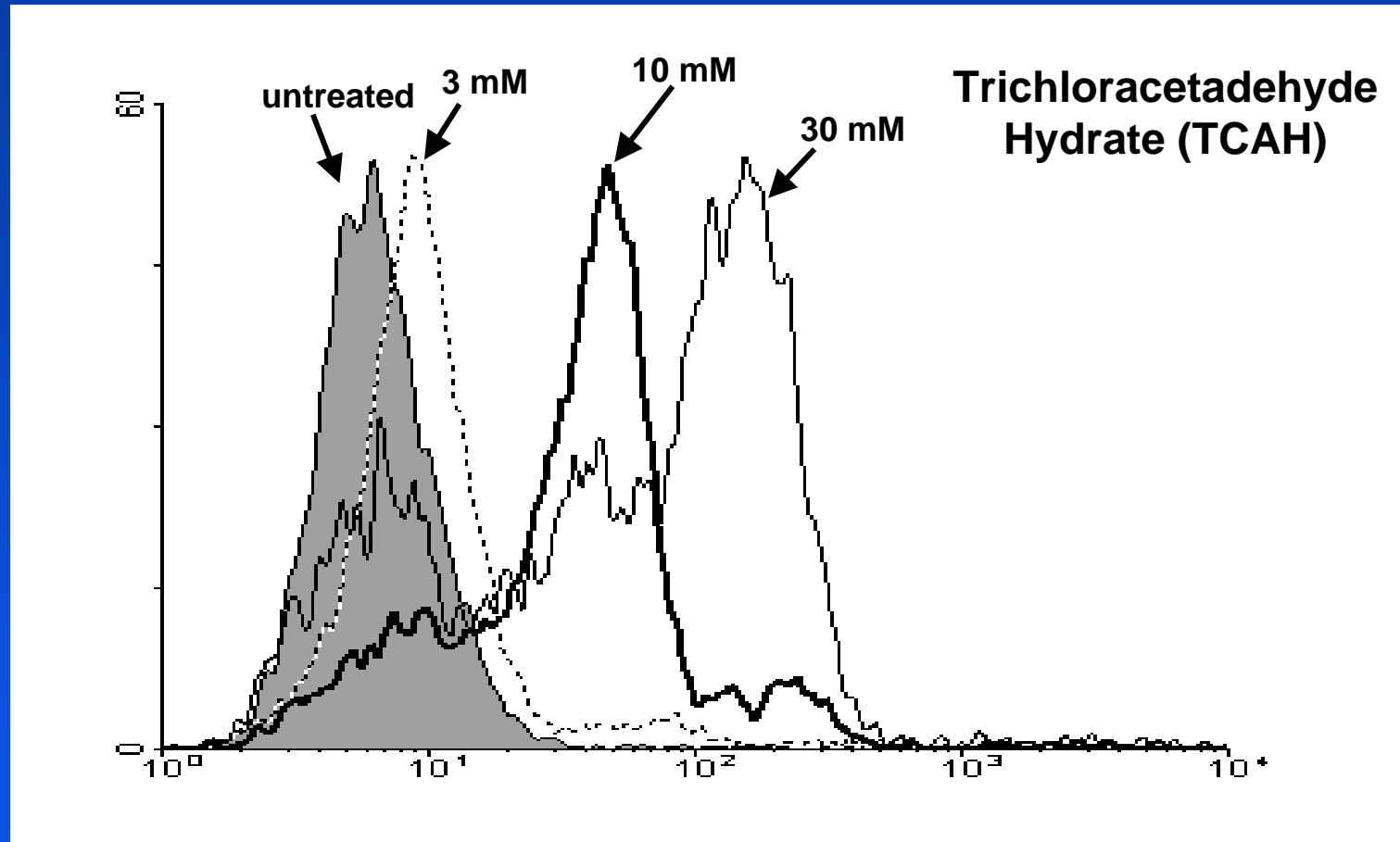
T Cell Activation by Forming a Schiff-Base



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

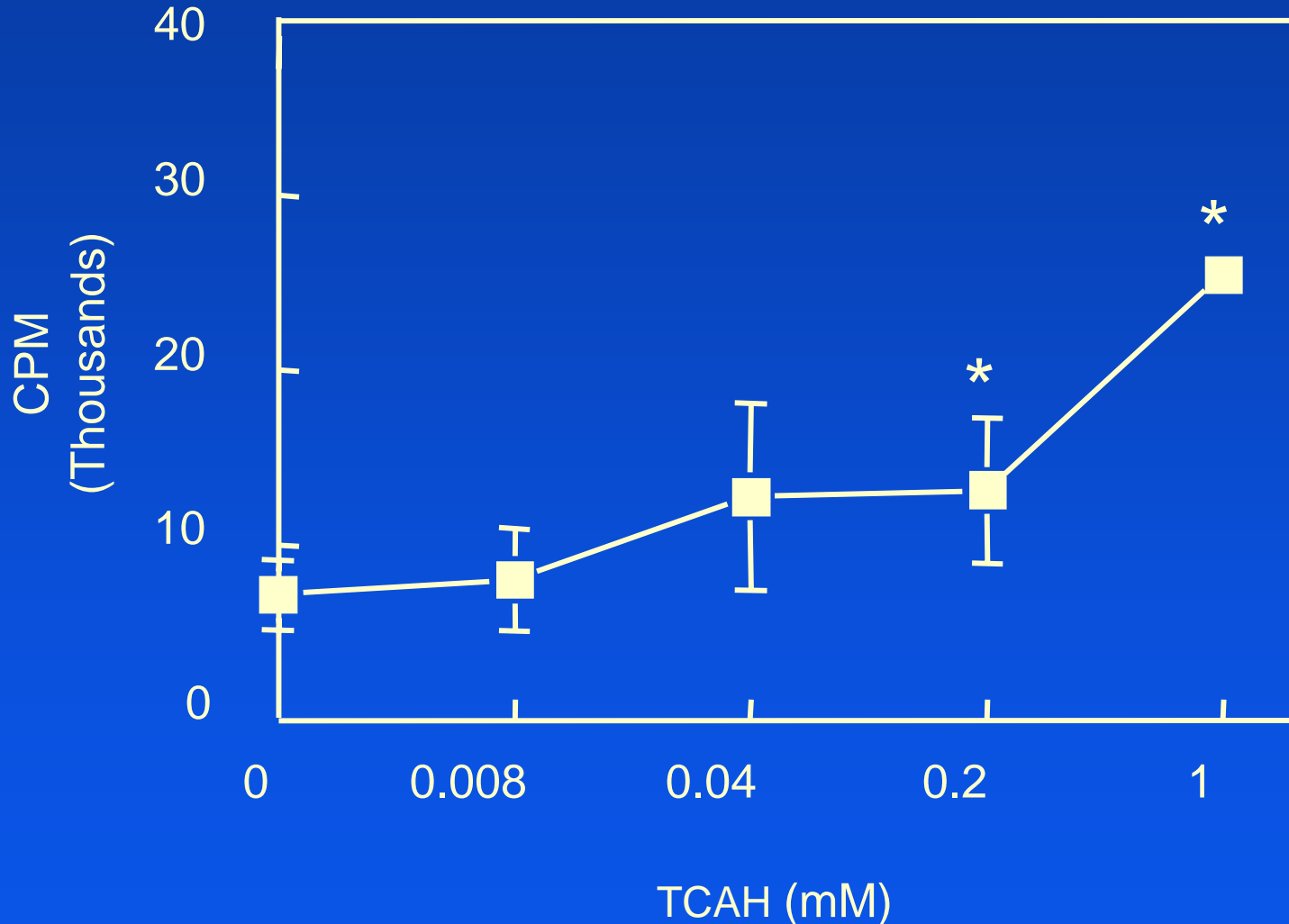


Trichloroacetaldehyde Binding to Th1 Cell Surface Protein



Fluorescence Intensity

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

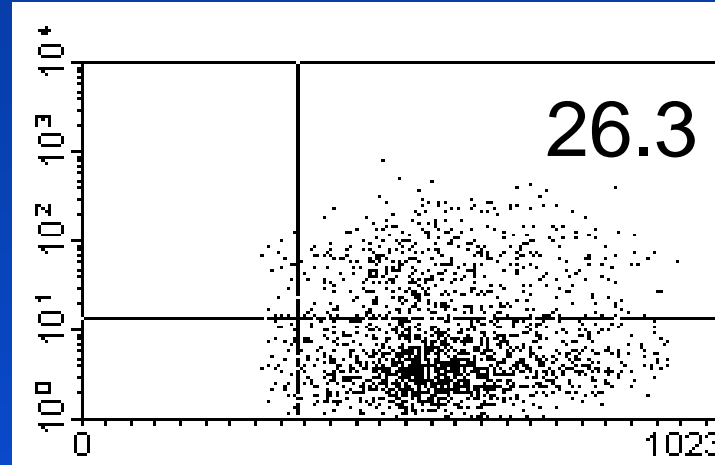
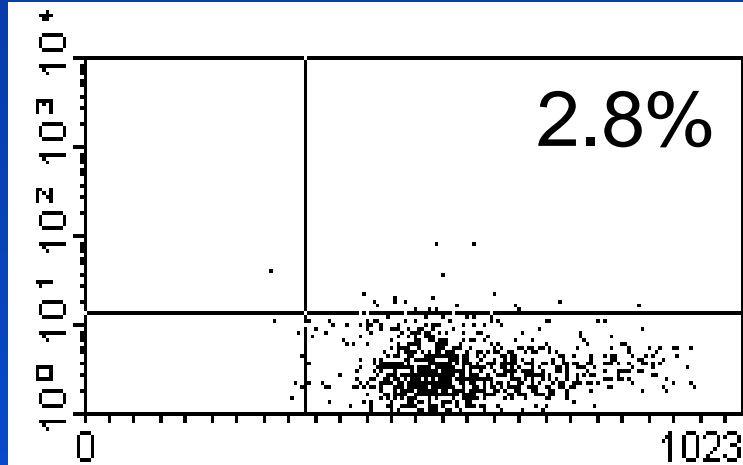


TCAH Stimulates Th1 Cells Activation

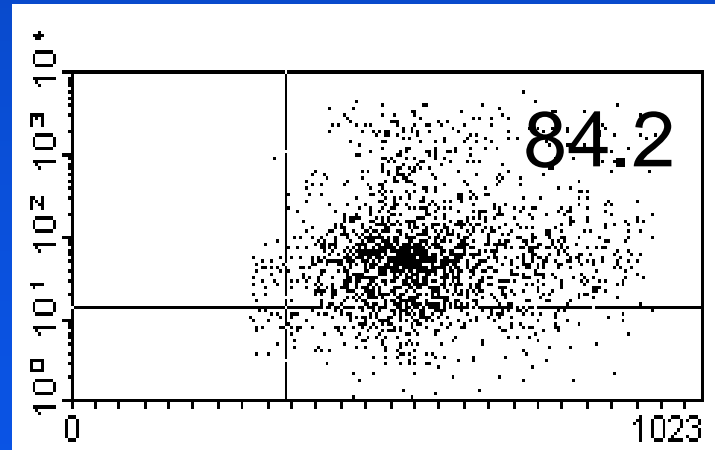
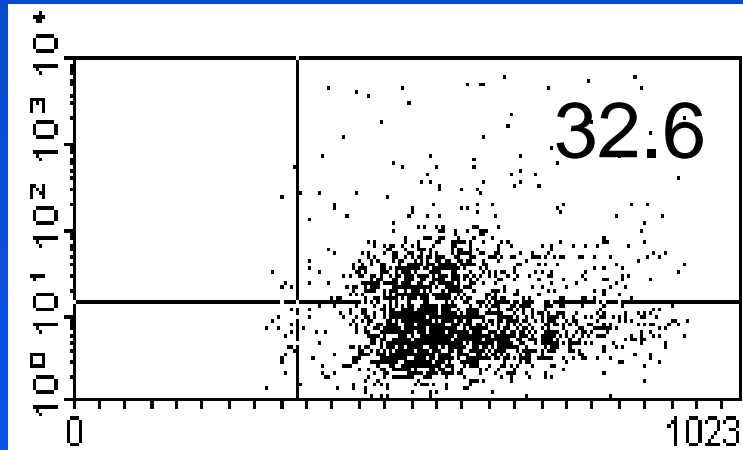
No Anti-CD3

Anti-CD3

CD28



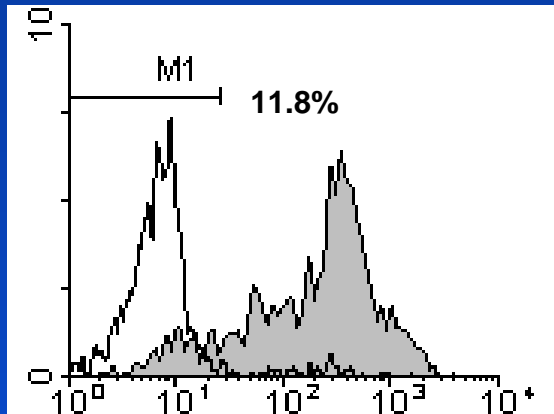
No
TCAH



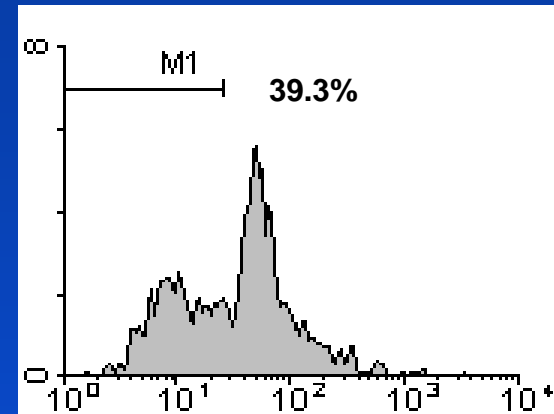
TCAH
(1 mM)

Trichloroacetaldehyde Hydrate (TCAH) Stimulates Activation (CD62L^{lo}) in CD4⁺ T Cells

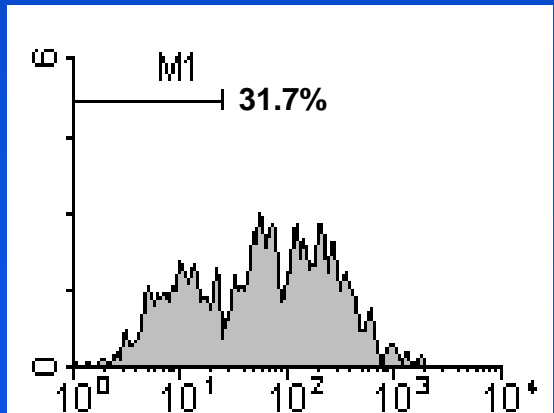
0 mM TCAH



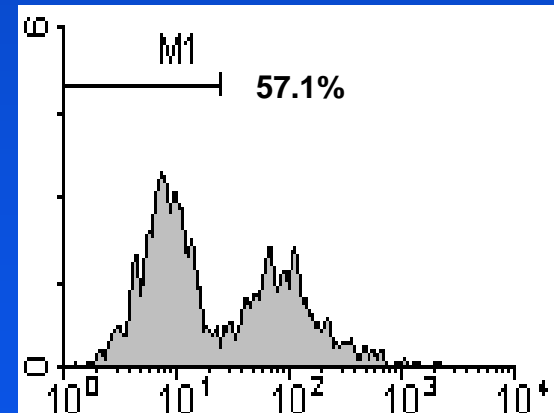
0.2 mM TCAH



0.04 mM TCAH



1 mM TCAH



Relative number CD62L^{lo}

Fluorescence Intensity

Fluorescence Intensity

Experimental Design

Autoimmune-Prone
MRL+/+ Mice



Trichloroacetaldehyde
Hydrate

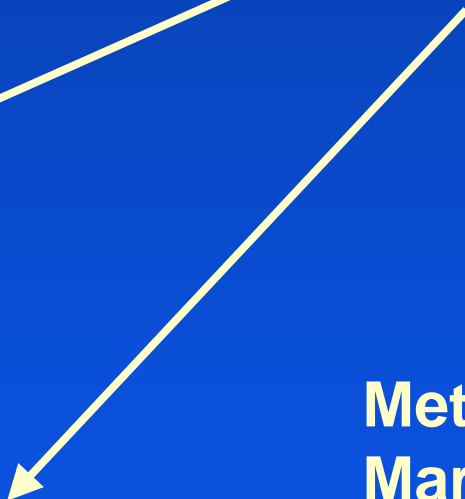


Serum
Spleen & lymph nodes
Liver & lungs

Toxicity
Autoimmune markers

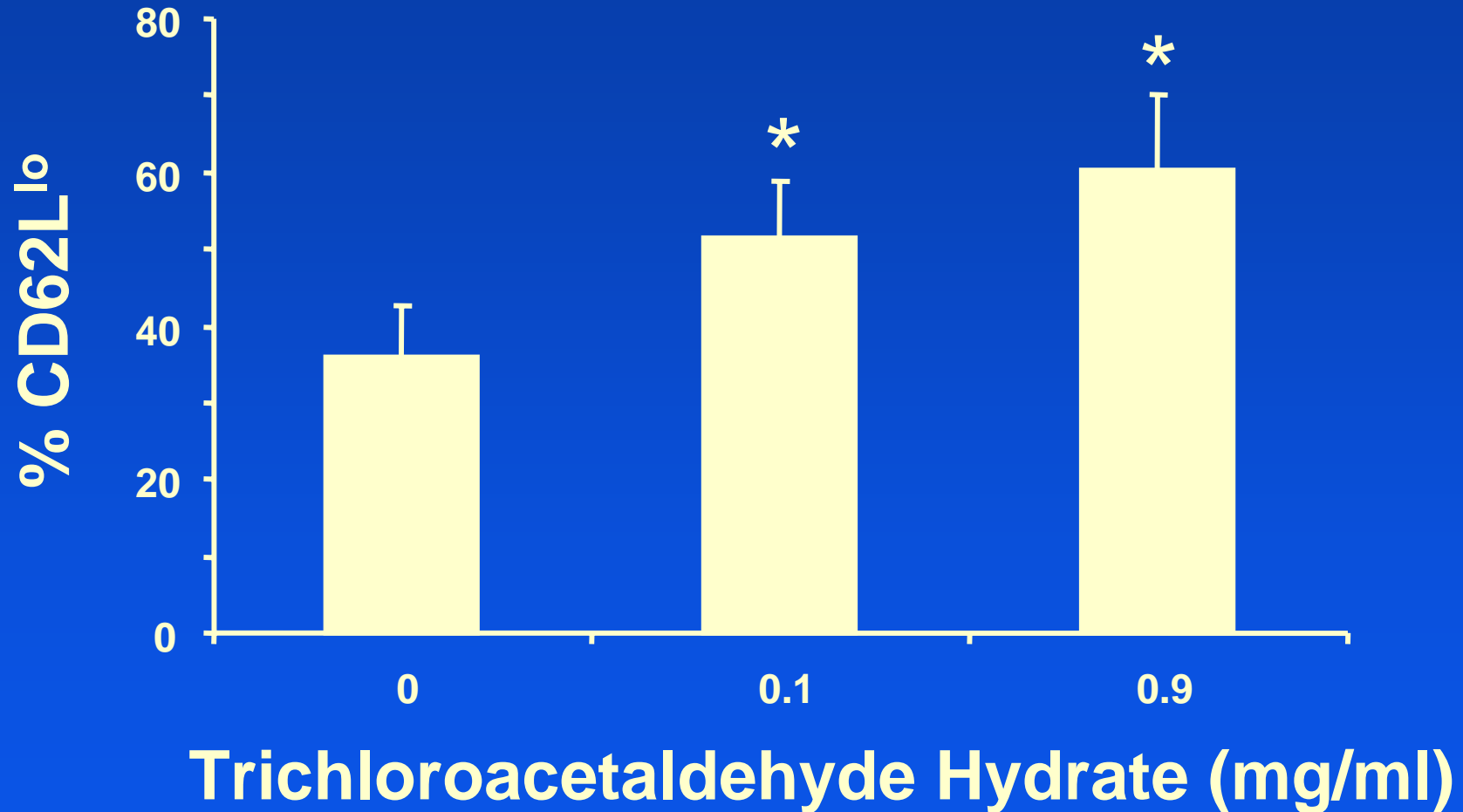
T-cell Activation
Cytokines (IFN γ & IL-4)

Metabolic Activation
Markers of Oxidative stress



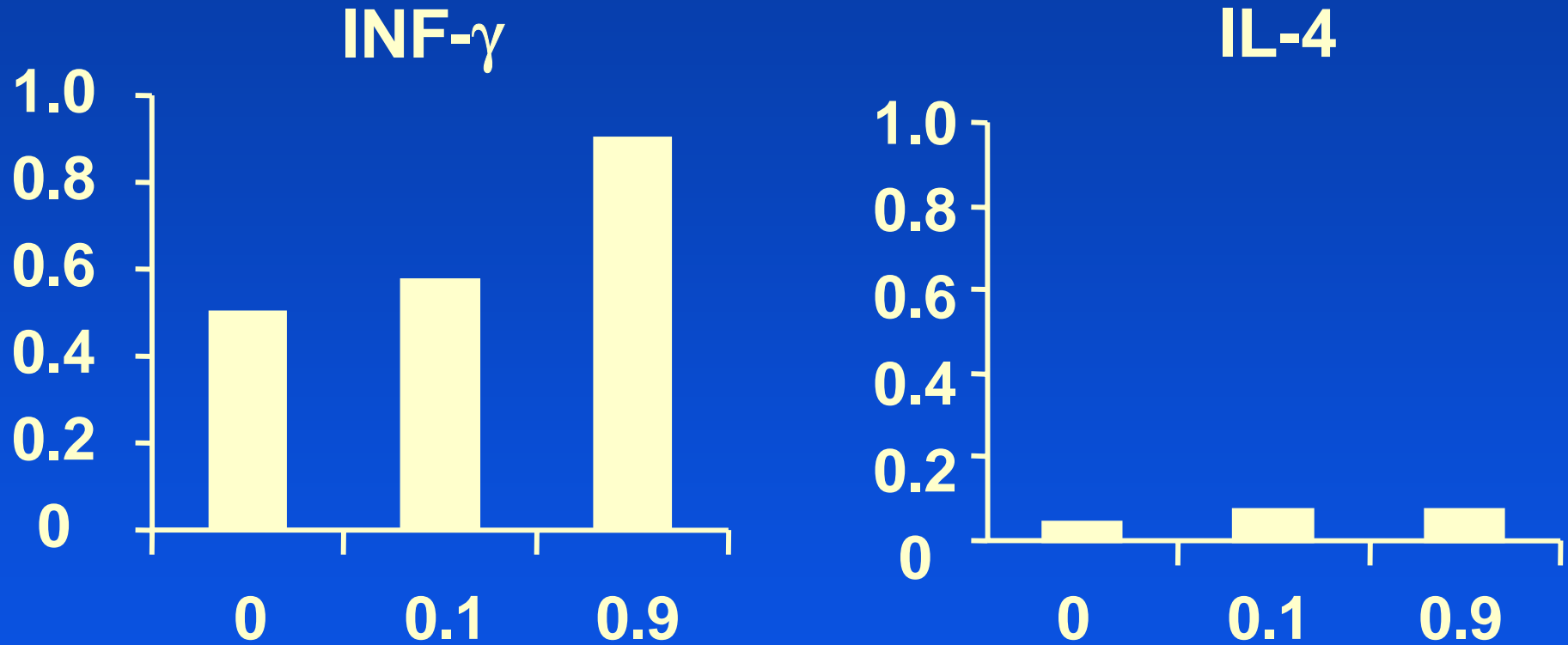
Expression of CD62L^{lo} on T cells

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



T cells Cytokine Secretion

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



Trichloroacetaldehyde Hydrate mg/ml

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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.