

# **The Role of Inflammation in the Safety Evaluation of Medical Device Materials**

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# Overview

## Exposure to endotoxin

- Exogenous (from medical devices) and endogenous (bacterial infection)
- Clinical importance of endotoxemia

## Interactive effects of endotoxin + chemicals

- Potentiation of toxicity by endotoxin
- Development of animal/*in vitro* models of endotoxemia/inflammation for toxicity testing

# Bacterial Cell Wall

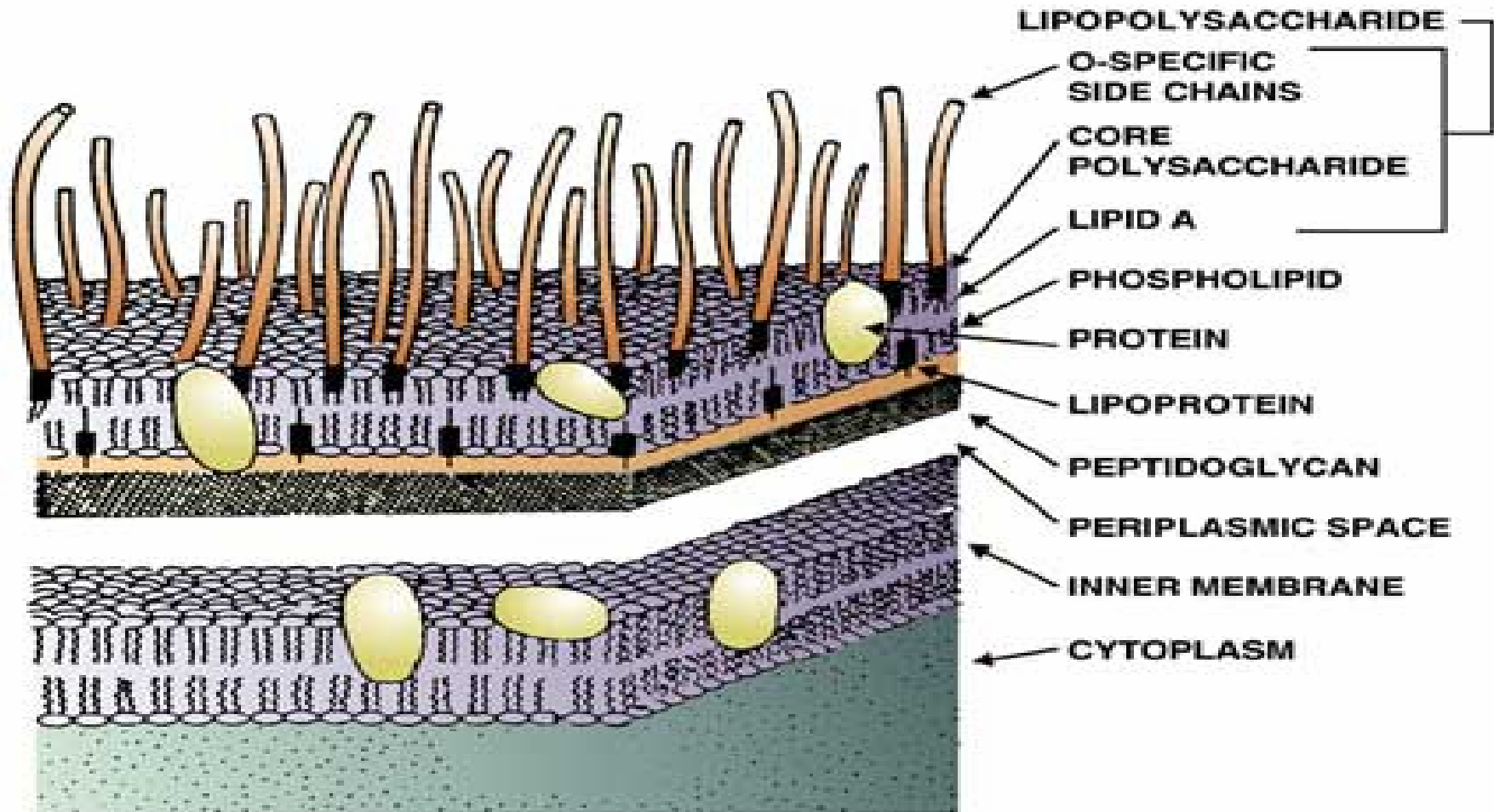


DIAGRAM OF A GRAM-NEGATIVE CELL MEMBRANE

# Sources of exposure to endotoxin

## Exogenous

- Improperly cleaned/contaminated medical devices



## Endogenous

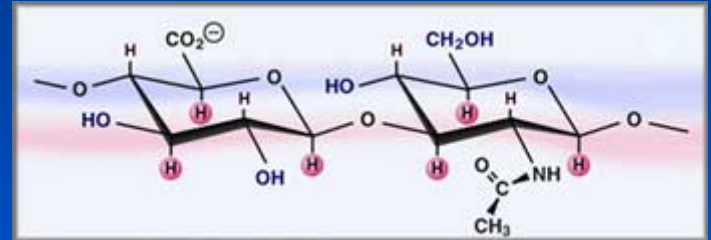
- Systemic infection
- Translocation from the gut



# Inflammatory response to hyaluronic acid preparation

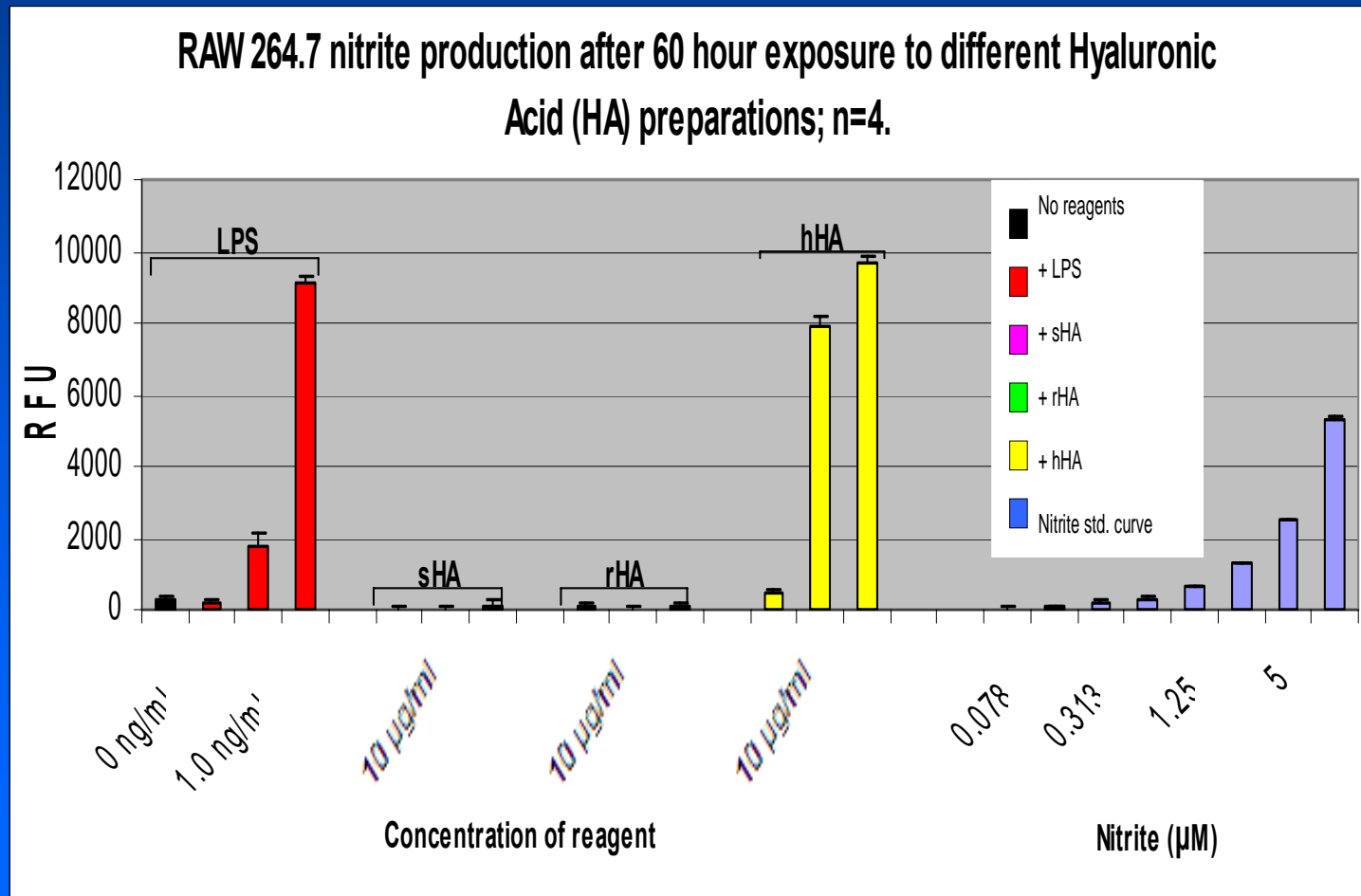
Dan Lyle, Jonathan Shallcross FDA, CDRH, OSEL

- Hyaluronic acid (HA) is a natural component of the body's extra-cellular matrix
- Medical device use: tissue filler to smooth skin wrinkles, biodegradable tissue engineering scaffolds
- Adverse events from injection of HA preparations have been reported, which may be inflammatory in nature.



# Inflammatory response to hyaluronic acid preparation

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# Inflammatory response to hyaluronic acid preparation

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**100 ug/ml HA**

**10 mg/ml HA**

**sHA**

**0.003 EU/ml LPS**  
**(0.005 ng/ml\* LPS)**

**0.3 EU/ml LPS**  
**(0.5 ng/ml\* LPS)**

**rHA**

**0.154 EU/ml LPS**  
**(0.257 ng/ml\* LPS)**

**15.4 EU/ml LPS**  
**(25.7 ng/ml\* LPS)**

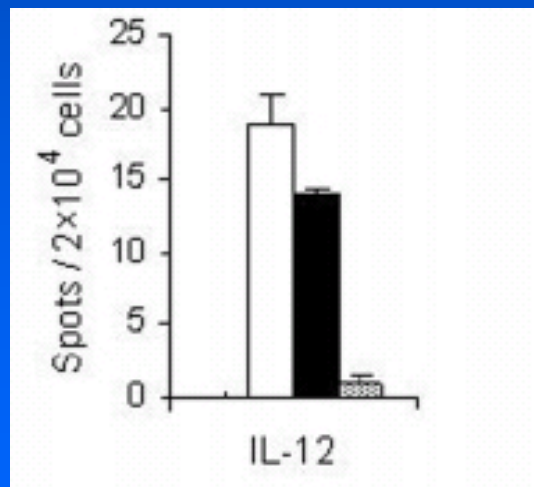
**hHA**

**92.1 EU/ml LPS**  
**(153.6 ng/ml\* LPS)**

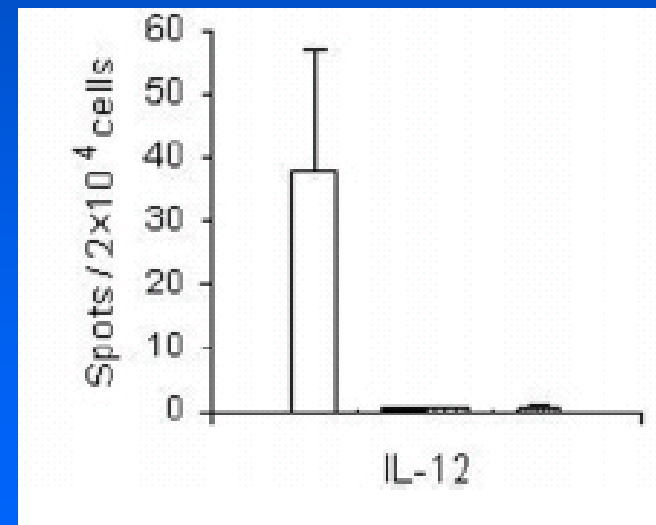
**9.2 x 10<sup>3</sup> EU/ml LPS**  
**(15.36 µg/ml\* LPS)**

# The Importance of an Endotoxin-Free Environment during the Production of Nanoparticles Used in Medical Applications

Helen Vallhov,<sup>\*,†</sup> Jian Qin,<sup>§</sup> Sara M. Johansson,<sup>†</sup> Niklas Ahlborg,<sup>‡</sup>  
Mamoun A. Muhammed,<sup>§</sup> Annika Scheynius,<sup>†</sup> and Susanne Gabrielsson<sup>†</sup>



**High LPS**



**Low LPS**

# Endogenous sources of exposure to endotoxin

- Sepsis is a severe illness caused by inflammatory response to toxin-producing bacteria.
- Response to LPS in Gram negative bacteria cell wall
- Many hospitalized patients become septic.



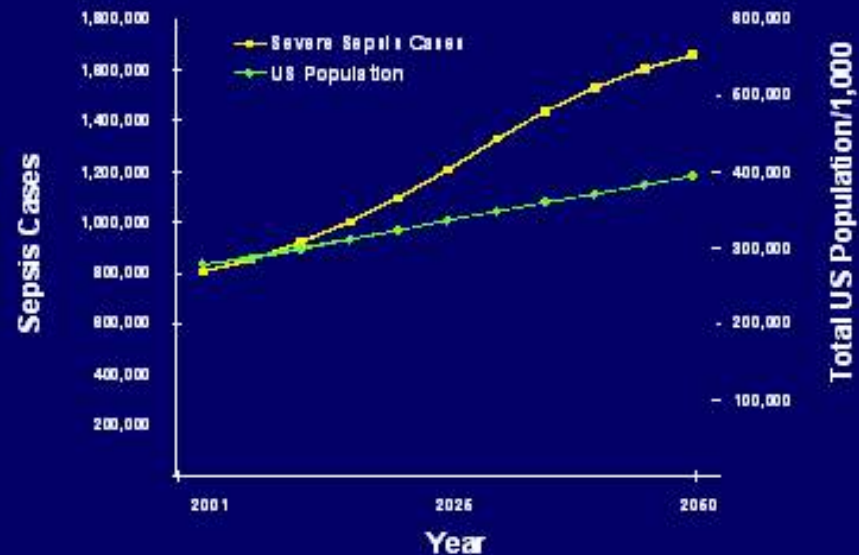
# Public health significance

## Severe Sepsis: A Growing Healthcare Challenge

Today

>750,000  
cases of severe  
sepsis/year  
in the US\*

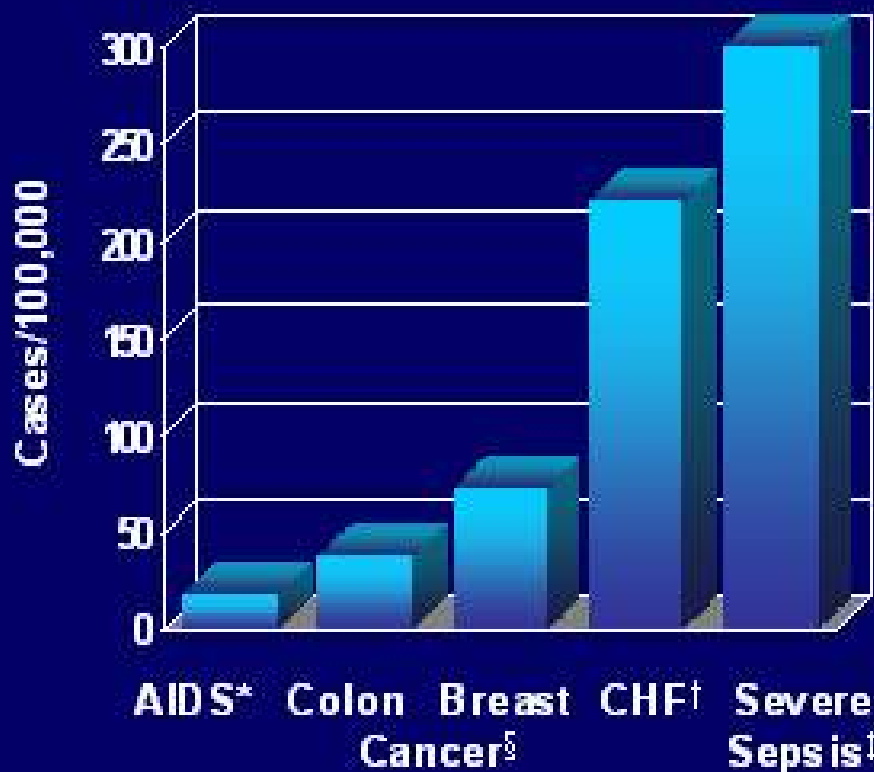
Future



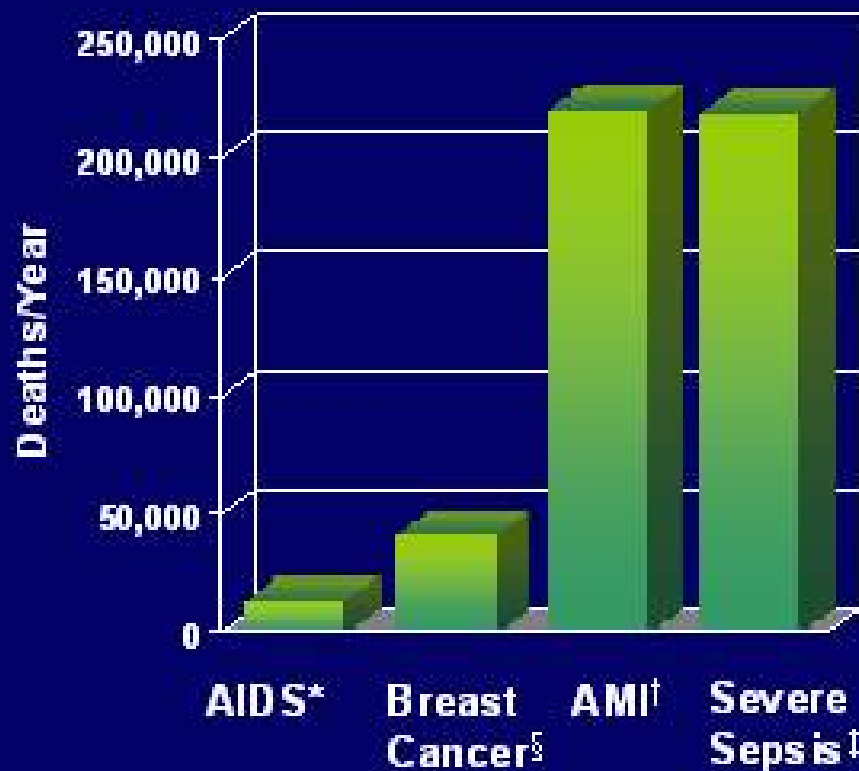
\*Angus DC. *Crit Care Med.* 2001 (In Press).

# Severe Sepsis: Comparison With Other Major Diseases

## Incidence of Severe Sepsis



## Mortality of Severe Sepsis



†National Center for Health Statistics, 2001. †American Cancer Society, 2001. \*American Heart Association, 2000. †Angus DC et al. *Crit Care Med.* 2001 (In Press).

# Increasing public awareness of sepsis



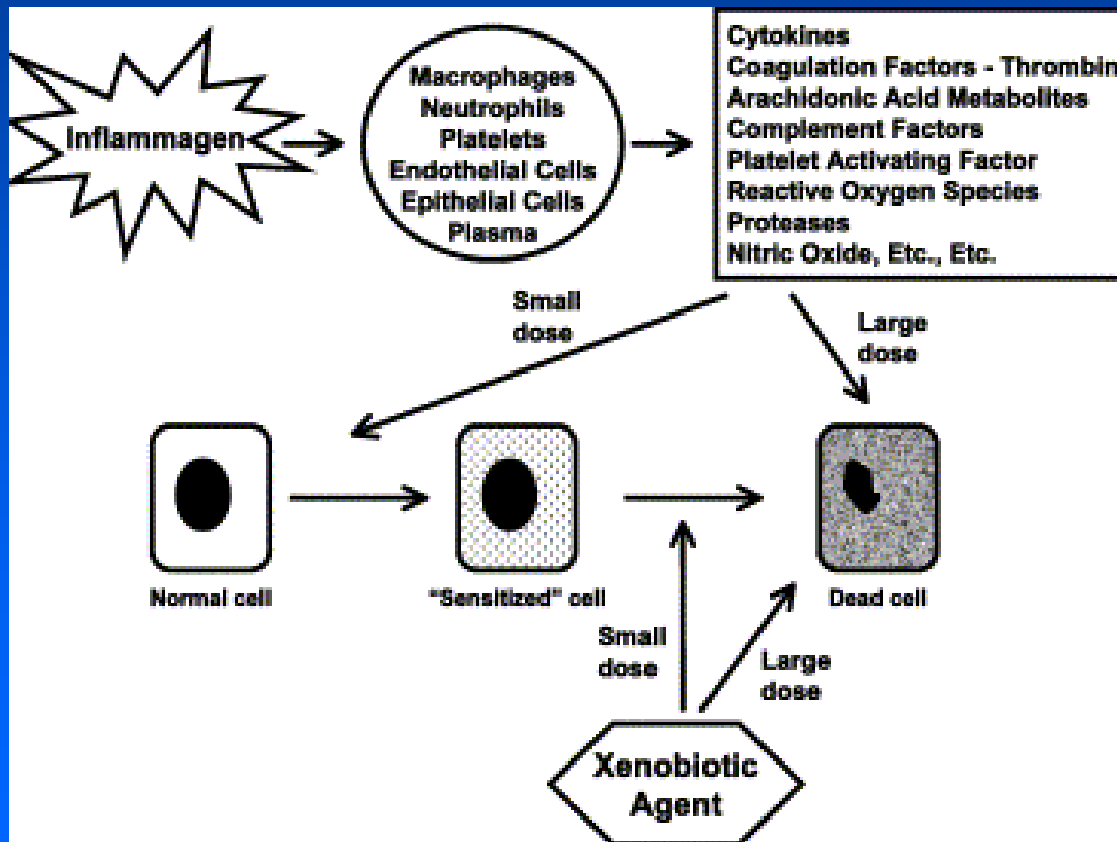
# Regulatory significance

- Septic patients are often treated with multiple medical devices
- Septic patients may be more susceptible than healthy individuals to effects from compounds released from medical device materials.

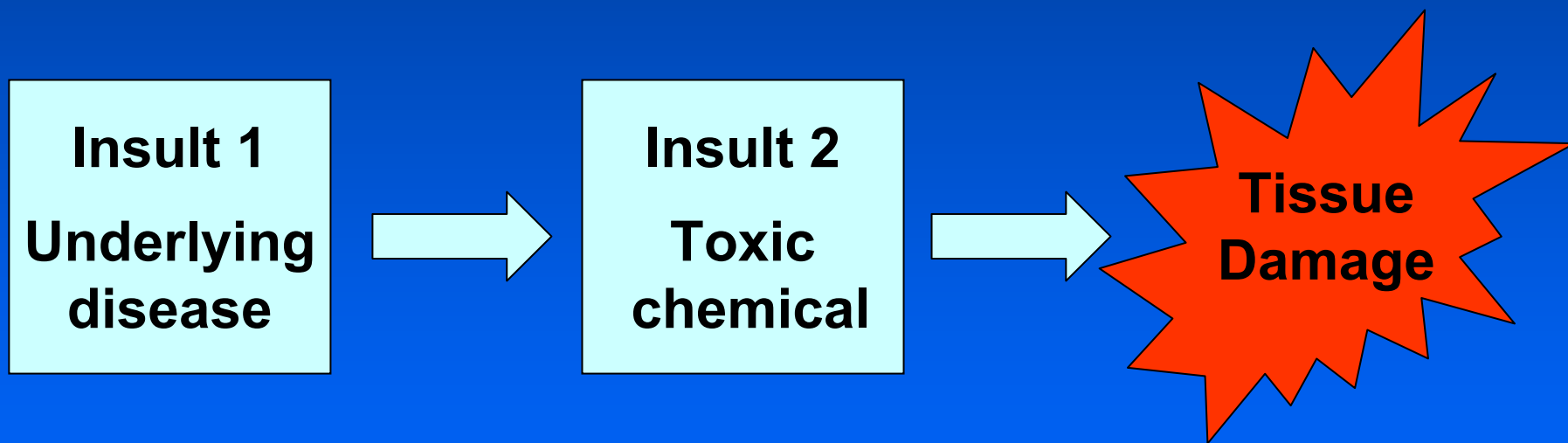


# Potentialiation of systemic toxicity by endotoxin

Ganey and Roth (2001) *Toxicology* 169(3):195-208

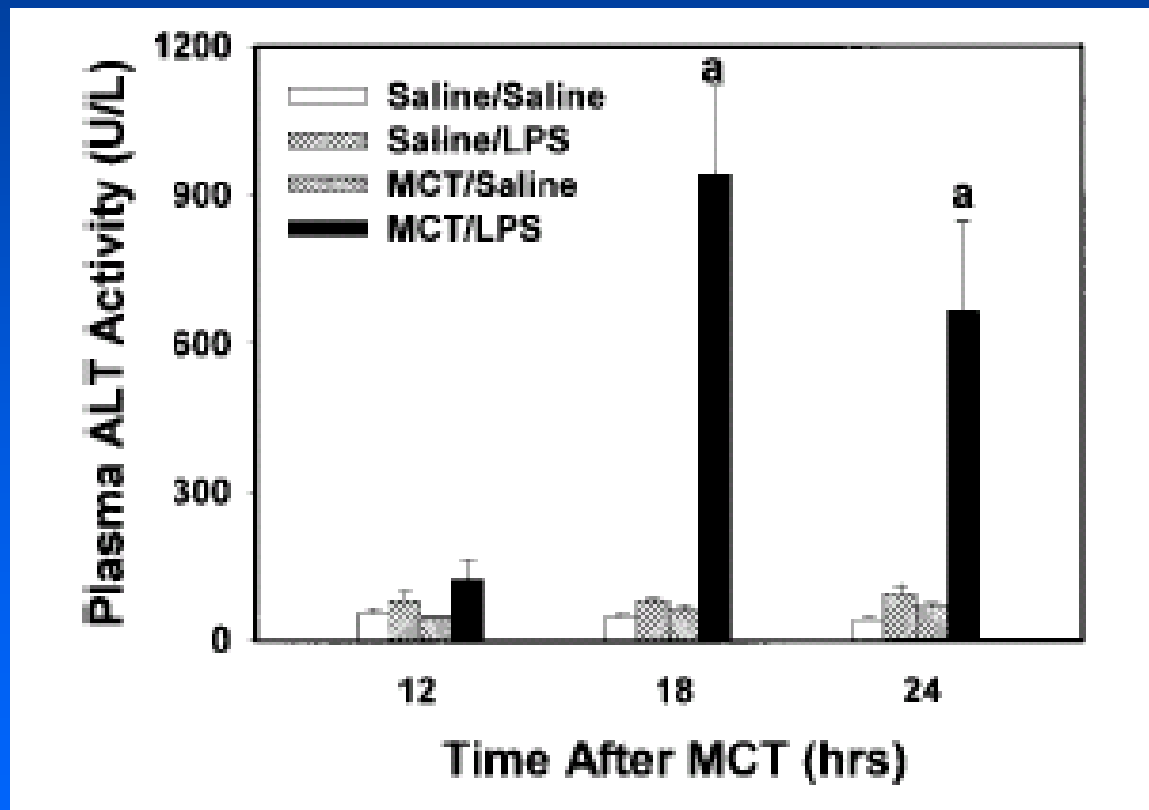


# “Two-hit” model of tissue injury



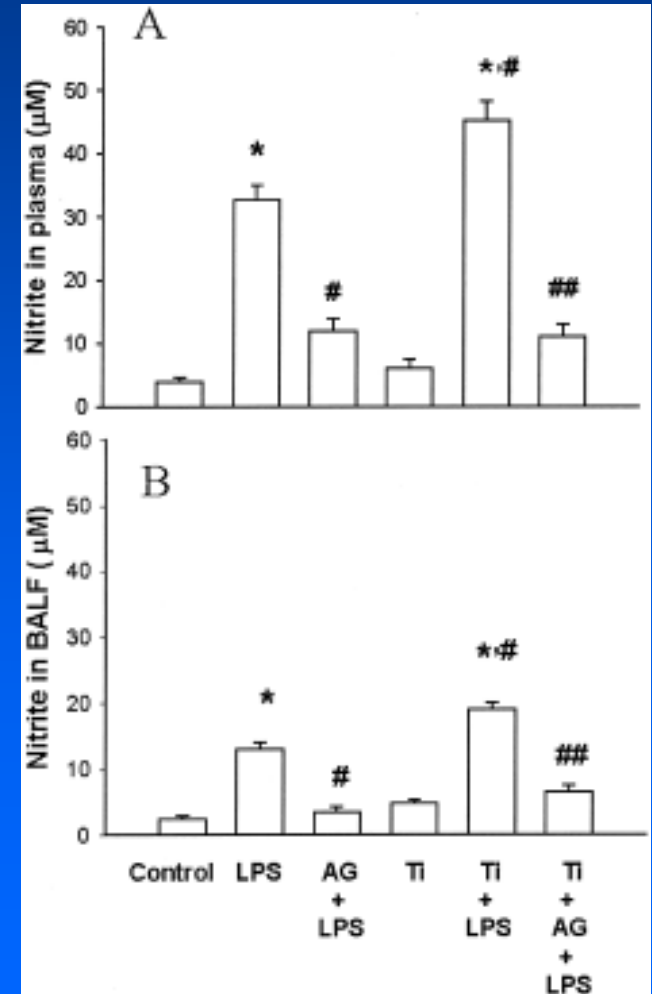
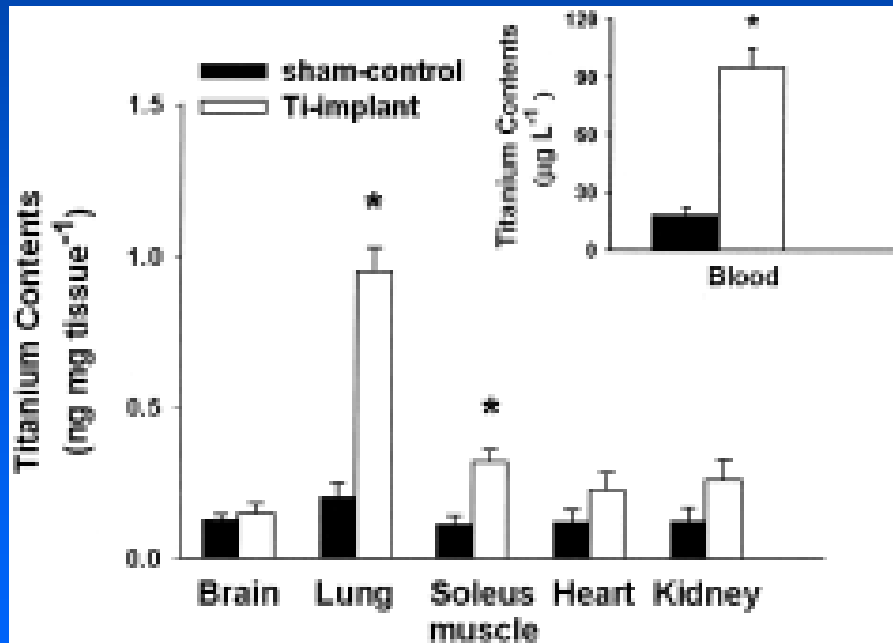
# Synergistic effect of LPS on monocrotaline hepatotoxicity in rats

Yee et al. *Toxicol. Appl. Pharmacol.* (2000) 166:173-185

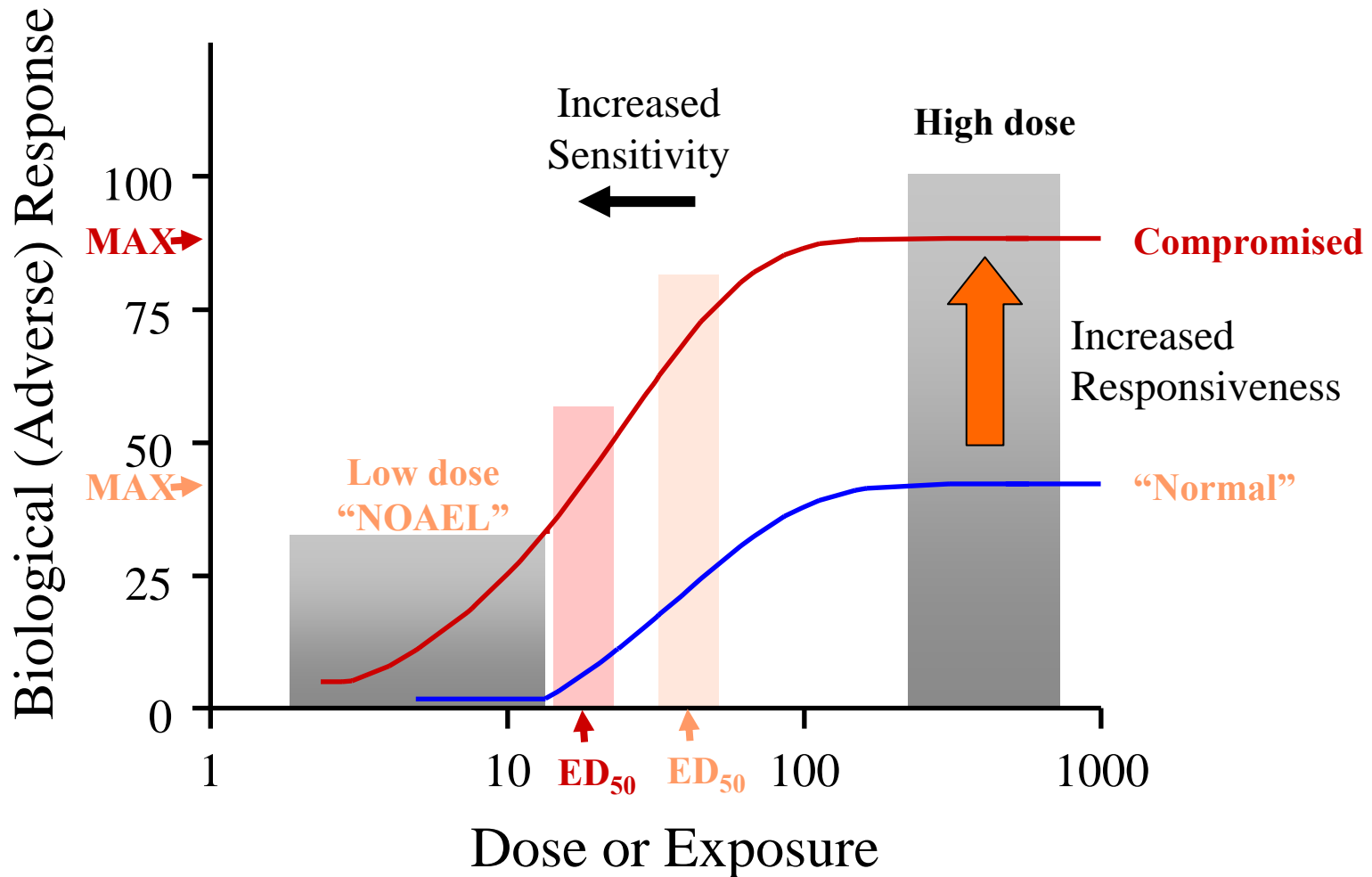


# Effect of titanium implants on pulmonary NO production in following exposure to endotoxin

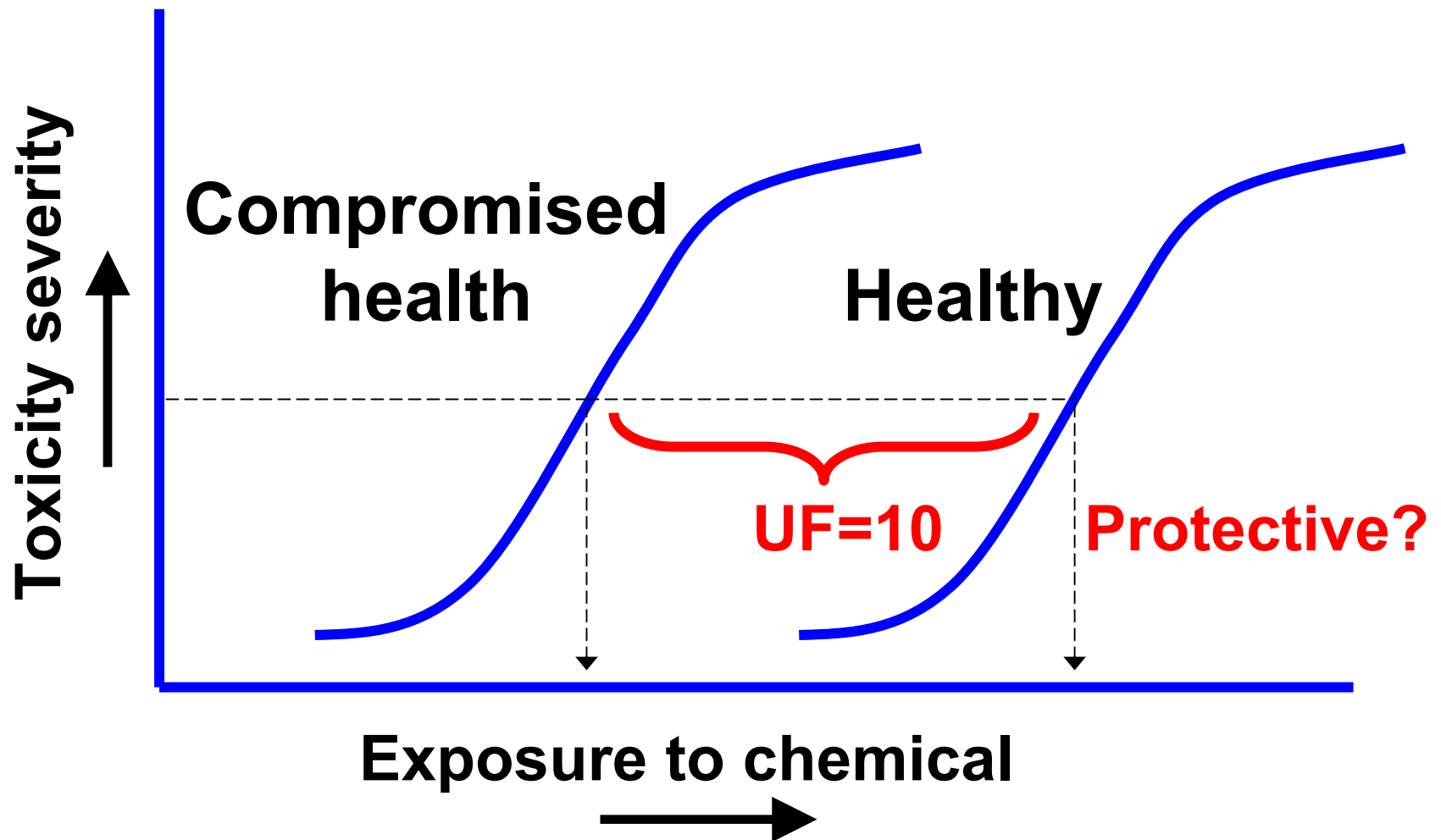
Yang et al. (2004) *J. Biomed. Mater. Res.* 69A:561-566



# Risks may be greater in health-compromised populations due to increased sensitivity or responsiveness to substances

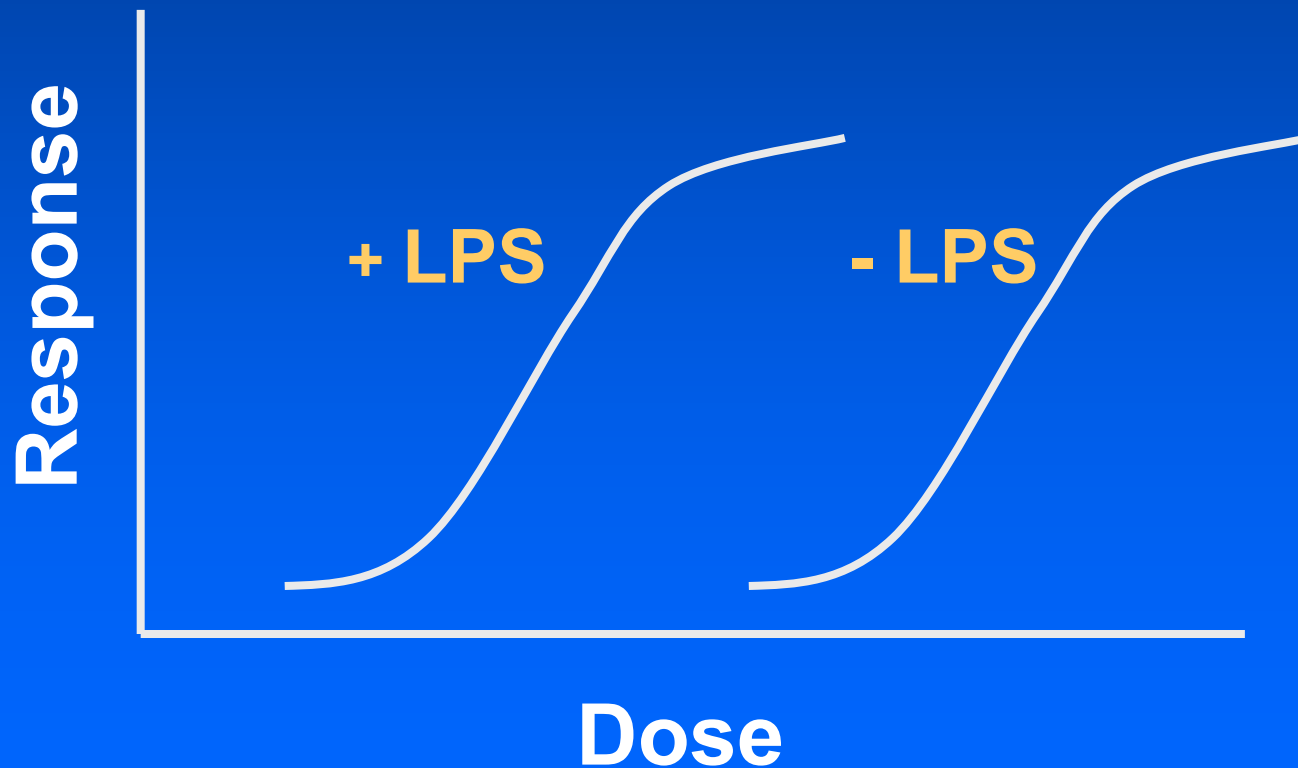


# Is the default uncertainty factor for interindividual variability adequate?



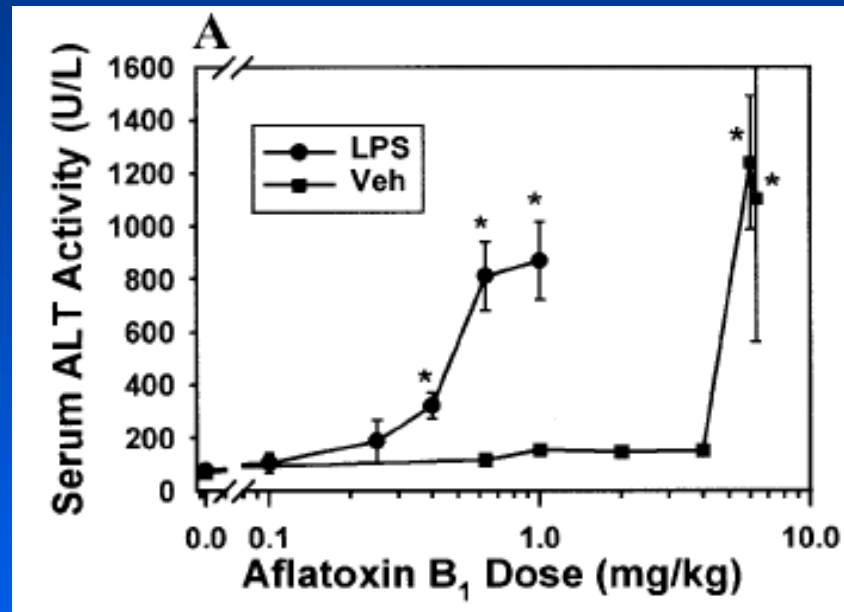
# Expected result: potentiation of chemical toxicity by endotoxin

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# Quantitative comparison of the extent to which LPS can potentiate toxicity

Luyendyk et al. (2002) *Toxicol. Sci.* 68(1):220-225



Effect of LPS Administration on AFB<sub>1</sub> Benchmark Doses

Serum enzyme	AFB <sub>1</sub> + Veh			AFB <sub>1</sub> + LPS		
	BMD (mg/kg)	95% CI <sup>a</sup>	GOF <sup>b</sup> p-value	BMD (mg/kg)	95% CI	GOF p-value
ALT	3.97	1.53	0.96	0.36	0.11	0.79
AST	3.71	1.57	0.89	0.38	0.08	0.90
ALP	4.61	3.59	0.39	0.18	0.10	0.88
GGT	ND	ND	0.81	0.22	0.16	0.58

# Inflammation studies: *In vivo*

## Animal models (Biocompatibility assessment and biomarker development)

- Rat model of endotoxemia
- Juvenile pig model of endotoxemia

## In vitro studies

- Anti-inflammatory effect of PVC extracts in LPS-stimulated RAW 264.7 macrophages
- Effect of PVC extracts on insulin release from pancreatic islet cells
- Inflammatory effect of hemoglobin
- Effect of hyperthermia on hemolysis

# Rat model of endotoxemia

Peter Goering, Ron Brown, FDA, CDRH, OSEL

## Initial results

- Reproducible model at 4- and 18-hour time points
- Hypothermia, weight loss, hyperlactemia, leukocytopenia, increased BUN and Cr

## Next steps

- Examine effect of compounds released from medical device materials on inflammatory response
- Identify early biomarkers of kidney injury in endotoxemic rats

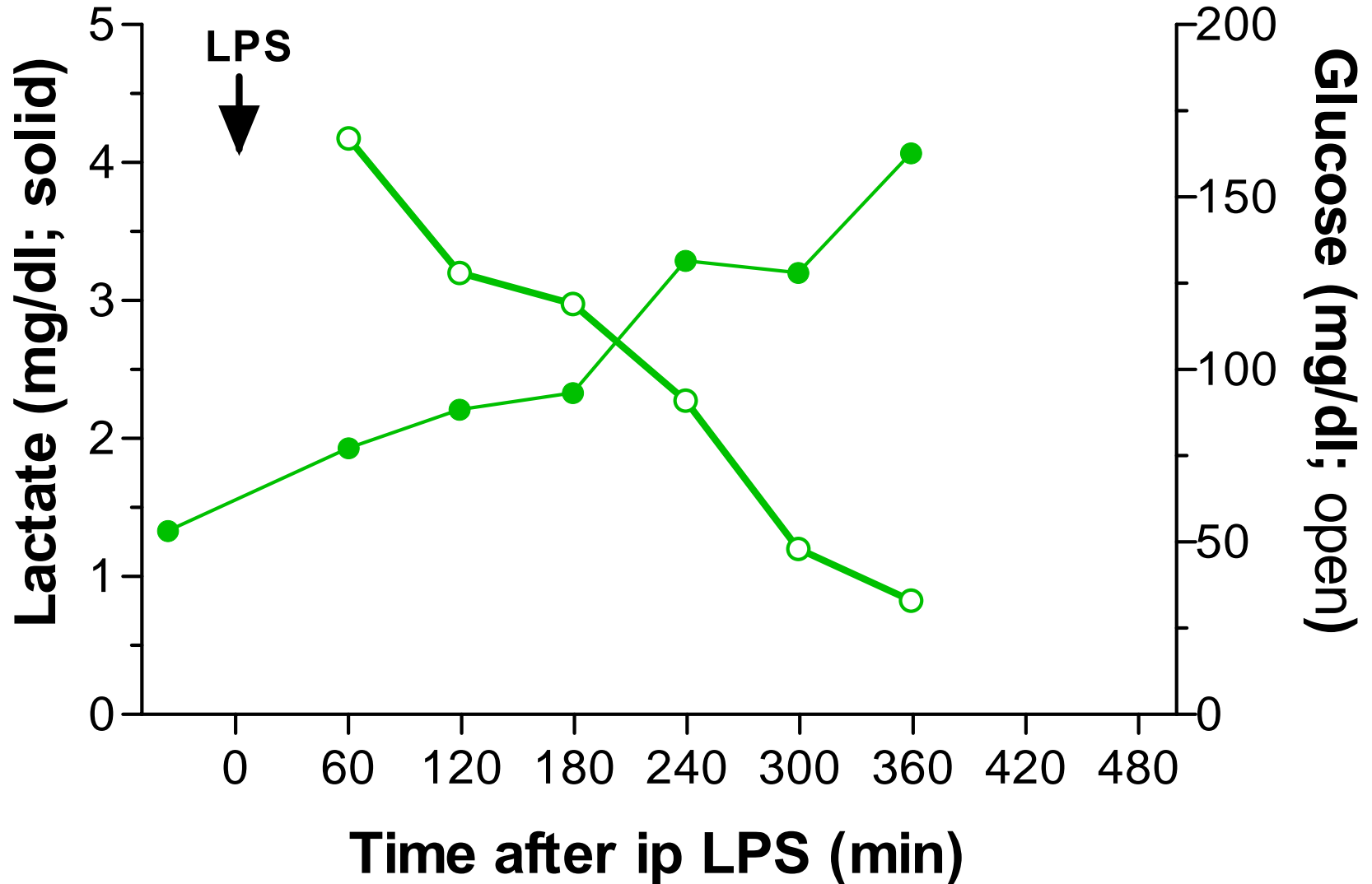


**Pig  
Model**

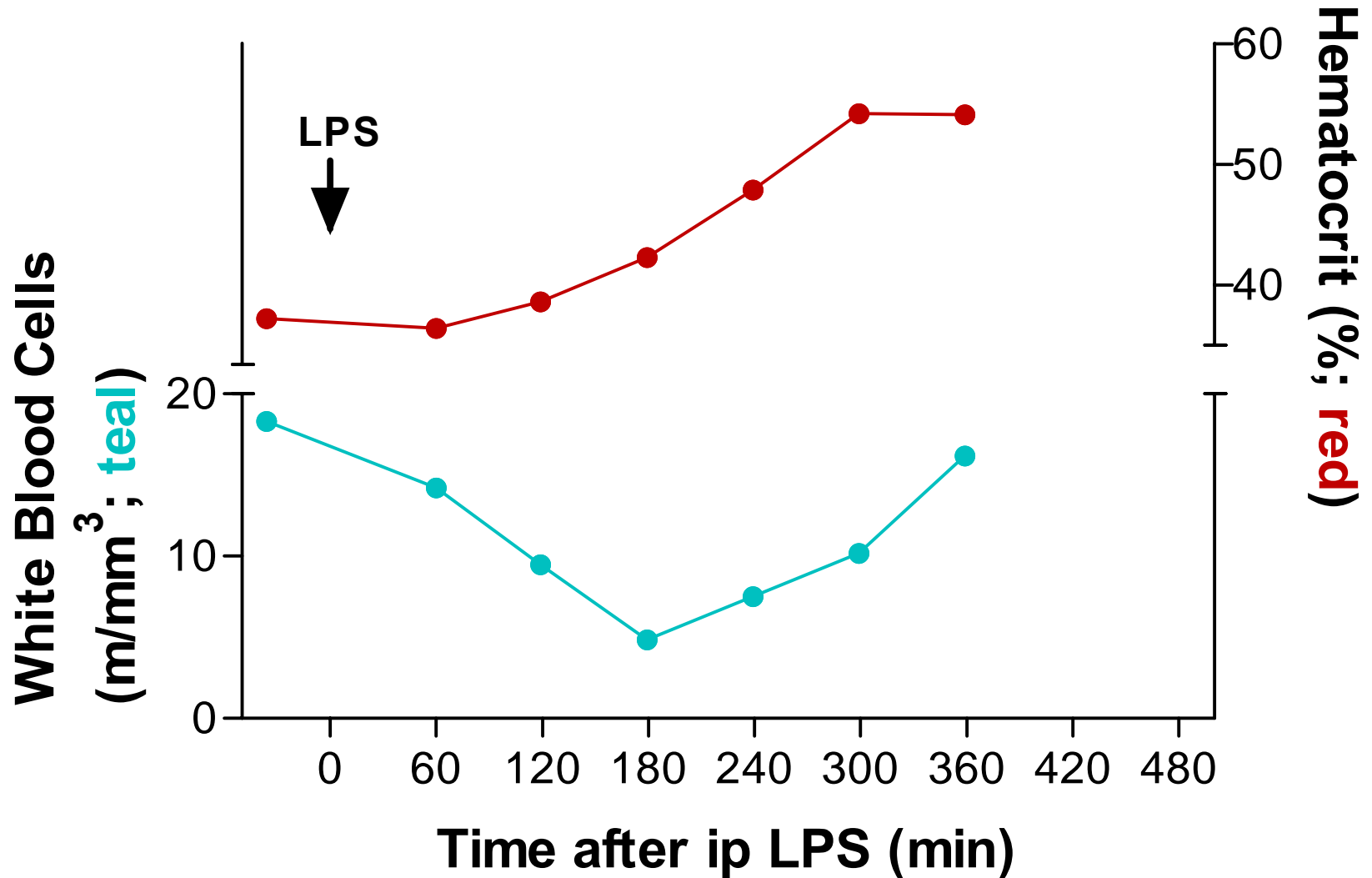
**Pediatric Patient**



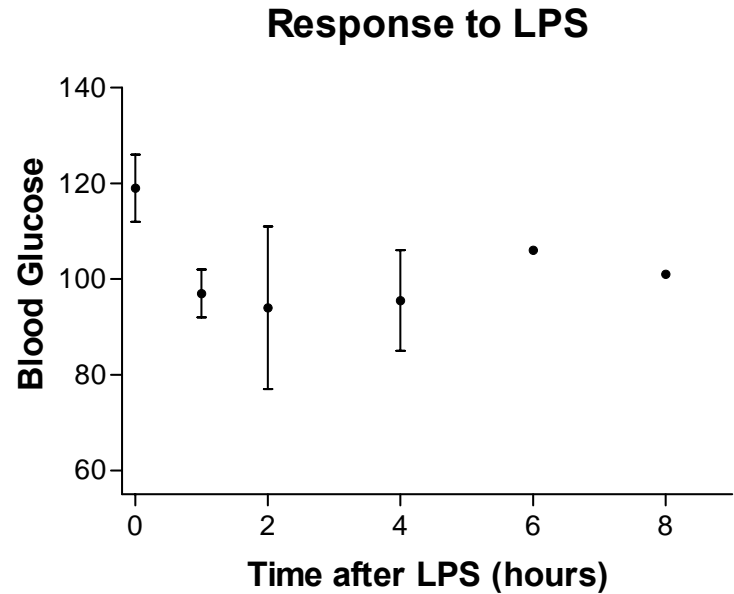
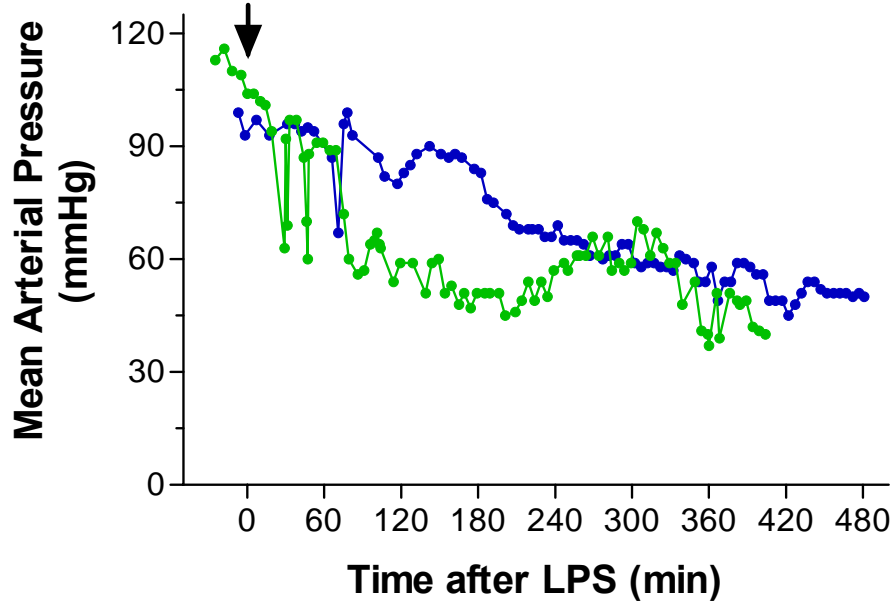
# Juvenile pig model of endotoxemia



# Juvenile pig model of endotoxemia



# Juvenile pig model of endotoxemia



# Inflammation studies: *In vitro*

## Animal models (Biocompatibility assessment and biomarker development)

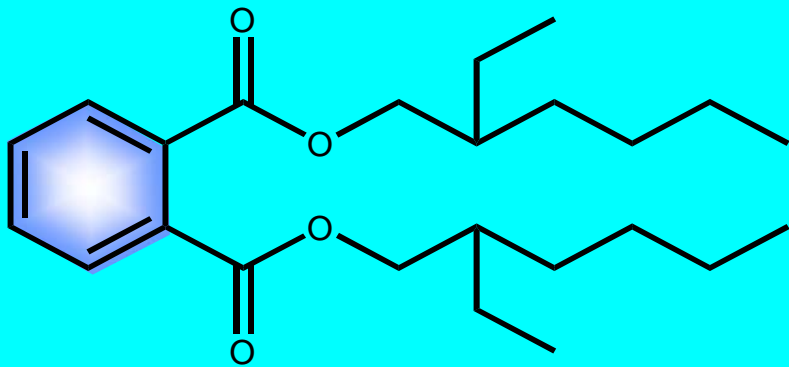
- Rat model of endotoxemia
- Juvenile pig models of endotoxemia

## In vitro studies

- **Anti-inflammatory effect of PVC extracts in LPS-stimulated RAW 264.7 macrophages**
- **Effect of PVC extracts on insulin release from pancreatic islet cells**
- **Inflammatory effect of hemoglobin**
- **Effect of hyperthermia on hemolysis/cytotoxicity**

# DEHP-containing medical devices

**DEHP: di(2-ethylhexyl) phthalate**



**Plasticizer used in PVC**

- Blood bags/tubing
- IV bags/tubing
- Hemodialysis tubing
- ECMO tubing
- Respiratory tubing
- TPN bags/tubing
- Enteral feeding bags/tubing



# Anti-inflammatory effect of PVC extracts and DEHP in LPS-stimulated macrophages

Qin Zhang, Ron Brown FDA, CDRH, OSEL

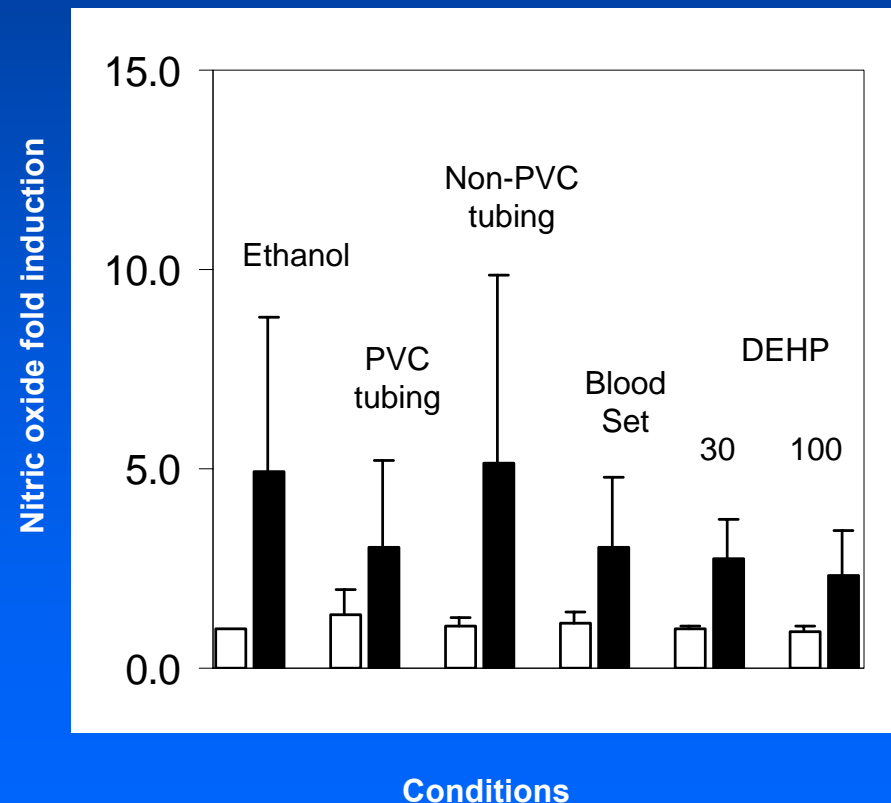
PVC and non-DEHP plasticized IV tubing extracted in ethanol

PVC blood administration set

DEHP at 30 and 100 ug/ml

Incubated with LPS-stimulated or control RAW 264.7 macrophages 24 hours

Interesting trend, but no significant difference



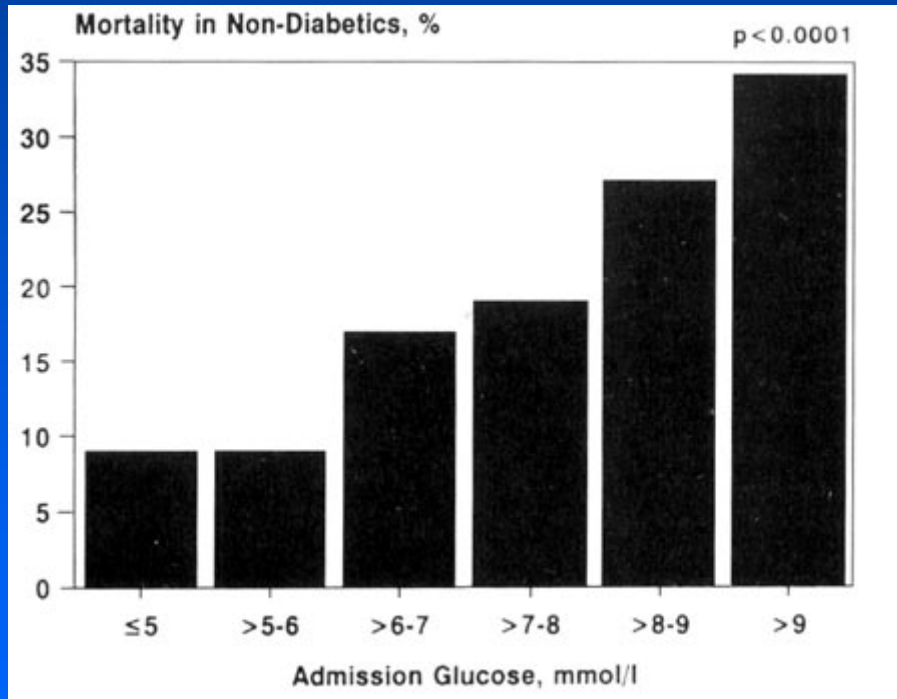
# Glycemic control in septic/critically ill patients

- **Hyperglycemia is common in septic and other critically ill patients**
- **Associated with adverse clinical effects**
- **Protocols in place to implement “tight glycemic control”**
- **Can compounds released from medical device materials contribute to the hyperglycemia seen in critically ill patients?**

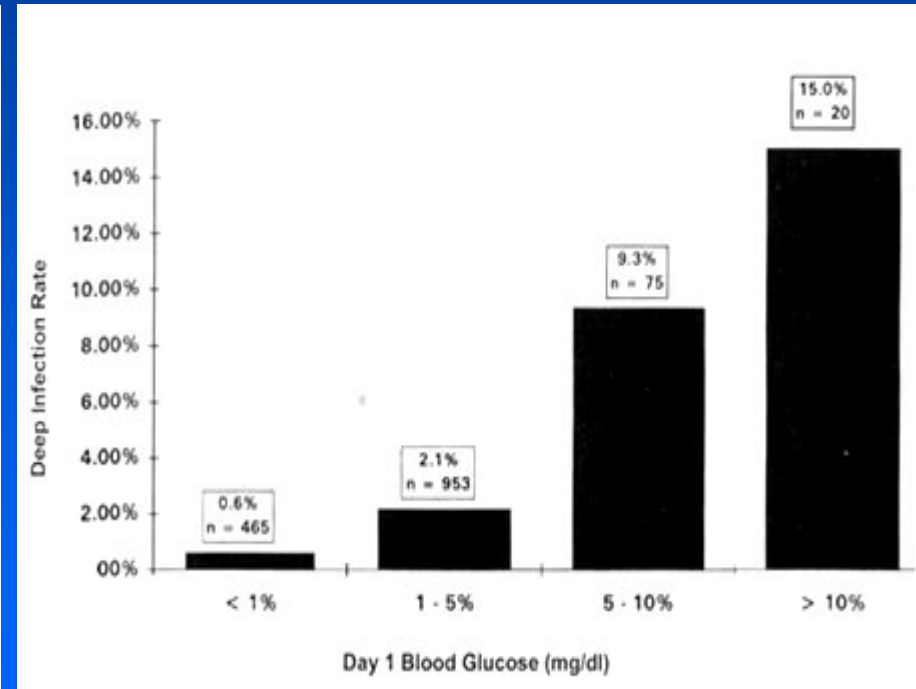
# The importance of hyperglycemia in the clinical outcome of hospitalized patients

**Levetan (2000) *Clinical Diabetes* 18(1):17**

## Mortality in stroke patients



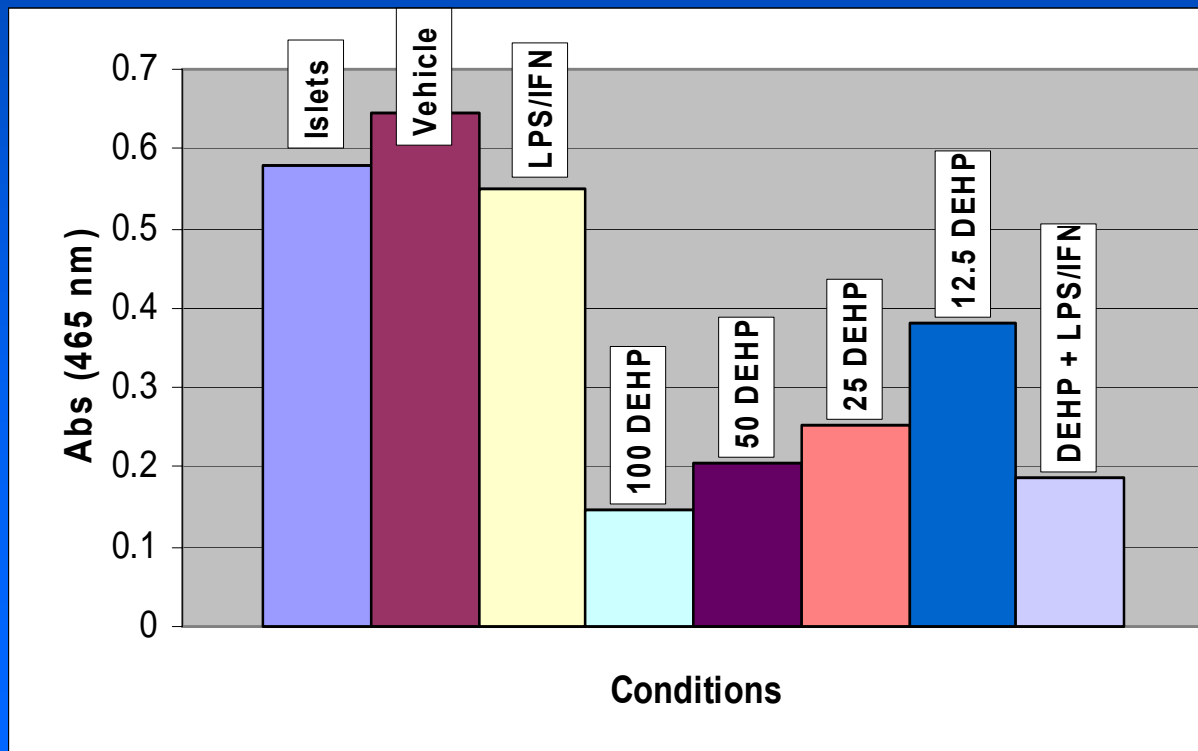
## Post-operative infection rate



# Role of DEHP in insulin release from rat islets

**Dan Lyle, Jonathan Shallcross FDA, CDRH, OSEL**

- DEHP/vehicle exposure x 20 hours
- 4 hour glucose challenge
- High glucose (400 mg/dl)
- Abs(456) corresponds to insulin level, measured by ELISA



# Effect of DEHP on glucose metabolism

Martinelli et al. (2006) *Hum. Exper. Toxicol.* 25:531-538

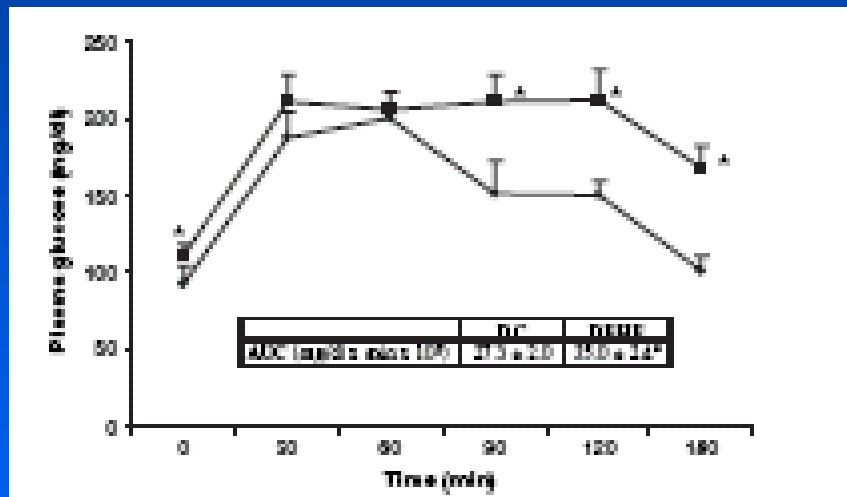
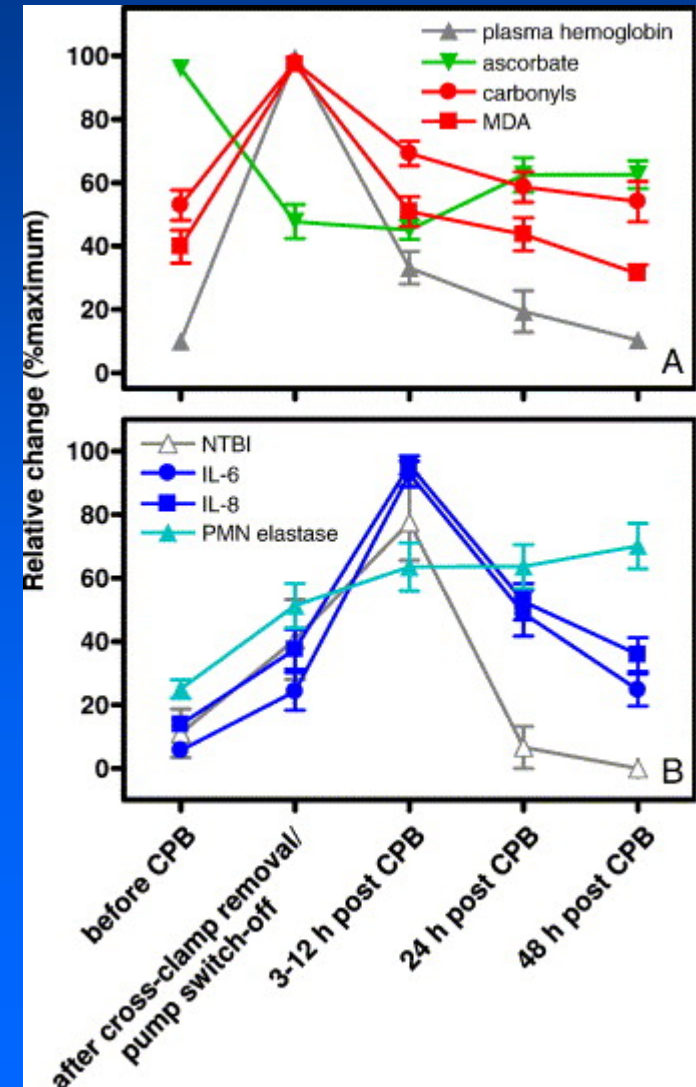


Table 1 Effect of DEHP on circulating metabolites

	Control	DEHP
Plasma glucose (mg/dL)	88 ± 7	114 ± 7**
Blood lactate (μmol/mL)	0.91 ± 0.07	0.90 ± 0.05
Blood pyruvate (μmol/mL)	0.04 ± 0.01	0.04 ± 0.01
Serum TG (mM)	0.42 ± 0.03	0.27 ± 0.02**

# Role of hemolysis as a mediator of inflammation in cardiopulmonary bypass

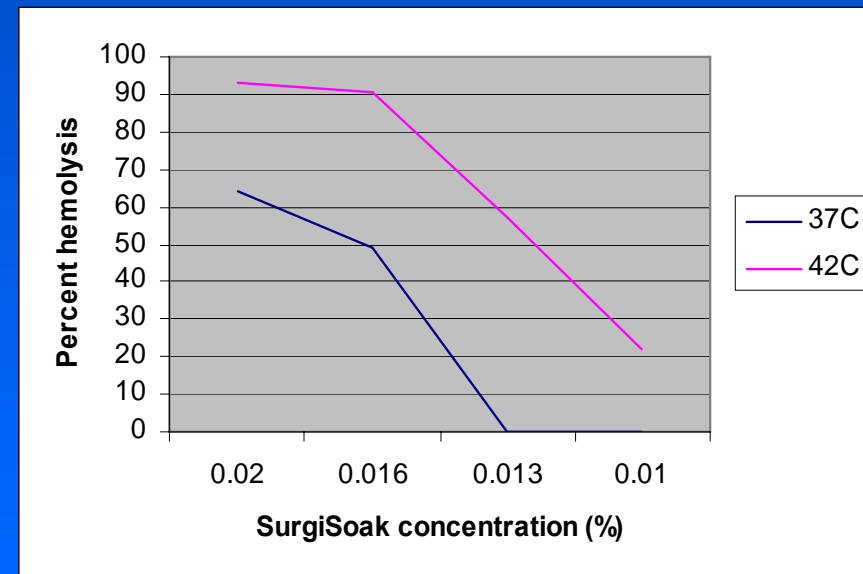
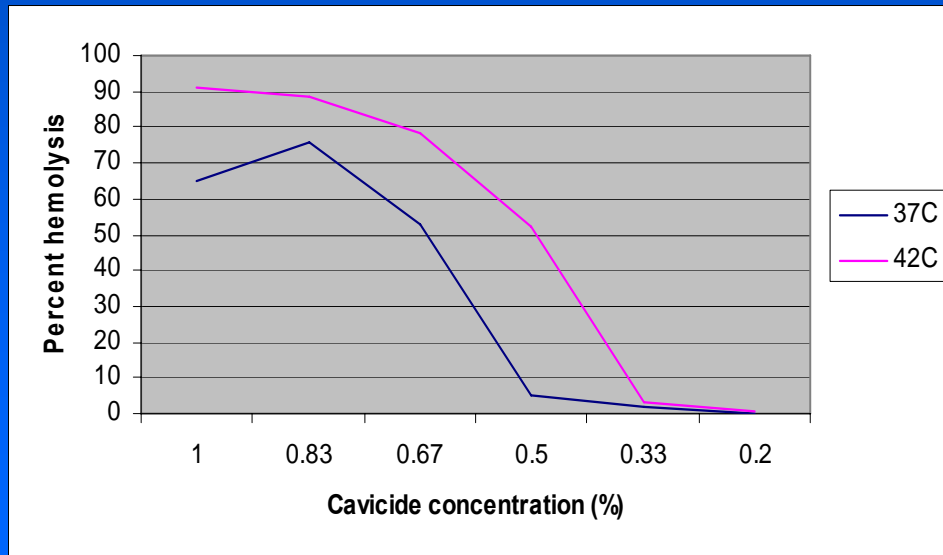
Christen et al. (2005) *Free Rad. Biol. Med.* 35:1323-1332



# Effect of hyperthermia on detergent- and disinfectant-induced hemolysis

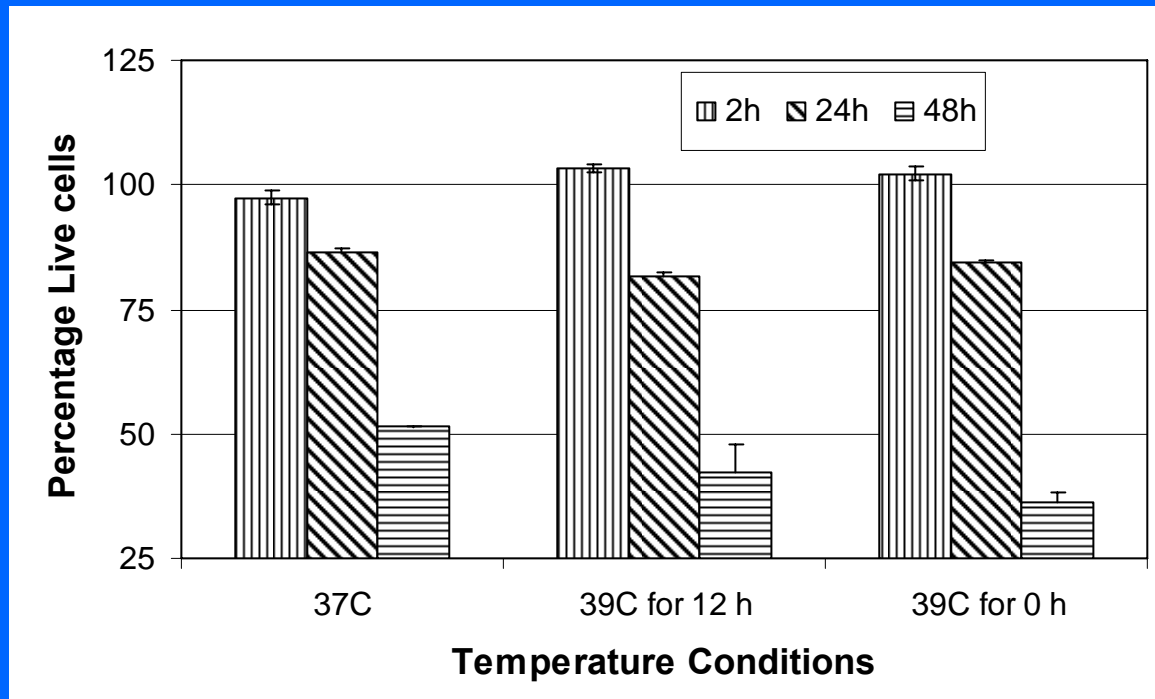
Yasmine Das, Ron Brown FDA, CDRH, OSEL

Incubation with cow RBCs for 24 hours at 37°C vs. 42°C



# Effect of mild hyperthermia (39°C) on the cytotoxicity of latex glove extracts in Jurkat cells

Lucas et al. (submitted) FDA, CDRH, OSEL



# *In vivo* potentiation of toxicity by hyperthermia

Endpoint	Species	Compound	Mean <sub>normothermic</sub>	Study
			LCL <sub>hyperthermic</sub>	
LD50	Mice	DXM	2.5	Muller and Vernikos-Danellis (1970)
LD50	Mice	Caffeine	1.6	Muller and Vernikos-Danellis (1970)
LD50	Rats	DDP	4.6	Wundergem et al. (1993)
LD50	Mice	Ethanol	2.1	Dinh and Gailis (1979)
LRR	Rats	Phenobarbital	1.4	Hisaoka and Levy (1985)

# Summary and Regulatory Implications

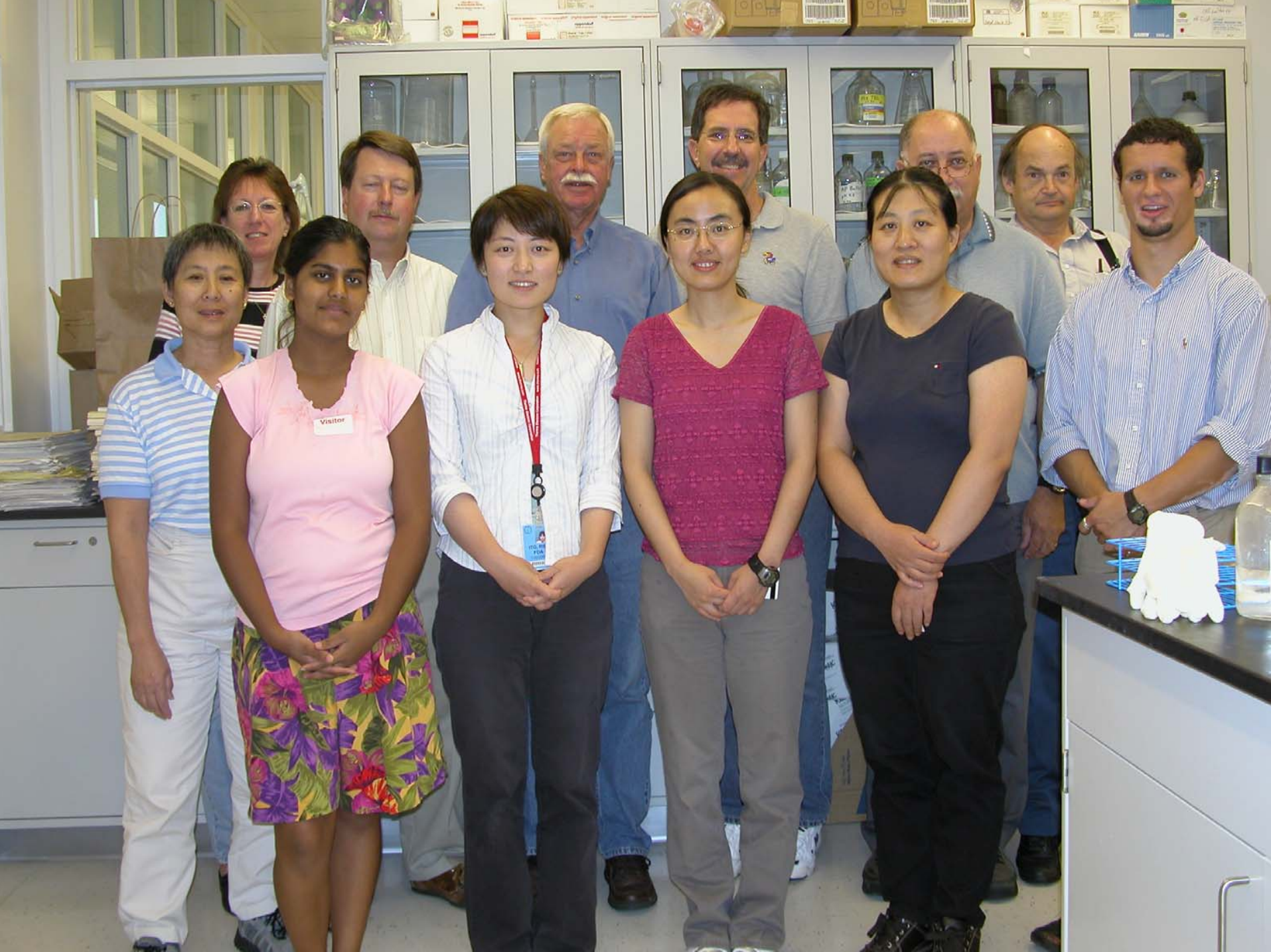
## Endotoxin contamination of materials

- **Some medical devices can be contaminated with endotoxin**
- **Inflammatory effect seen with some medical device materials may be produced by residual endotoxin**
- **Emphasizes the need for endotoxin limits and need for preclinical testing to assess inflammatory potential of some devices**
- **CDRH Immunotoxicity Test Guidelines:**  
<http://www.fda.gov/cdrh/ost/ostggp/immunotox.html>

# Summary and Regulatory Implications

## Potential of toxicity by increased inflammatory state

- Inflammation/hyperthermia can potentiate the toxicity of some medical device materials
- Animal/*in vitro* models of inflammation may be useful for preclinical testing of some medical device materials (e.g., intraperitoneal hydrogels) and for risk assessment (benchmark dose modeling, magnitude of default UF)





Ed Gordon