

Responses to Phthalate Exposure: Biological and Social Perspectives



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Outline

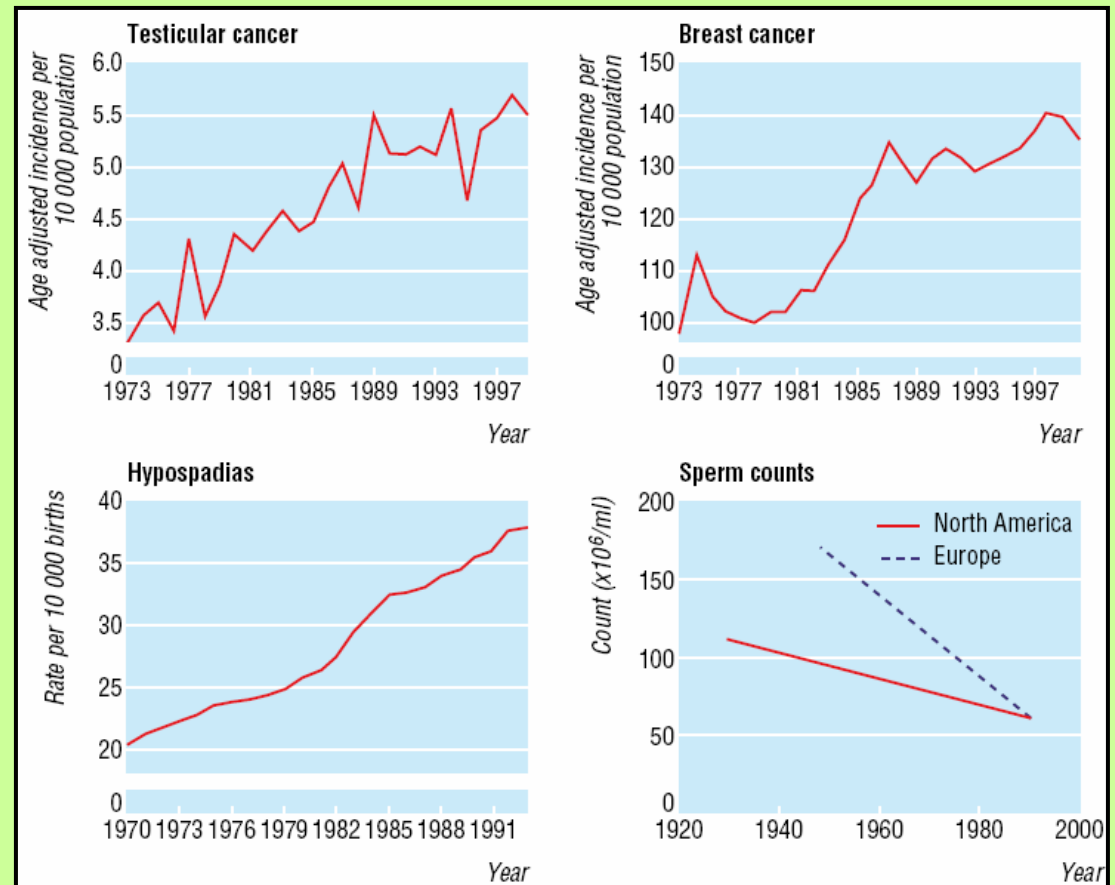
- Part I: Biological Responses to Phthalate Exposure
 - Background
 - Experimentation
 - Results
 - Conclusions
- Part II: Medical Responses to Phthalate Exposure
 - Background
 - Understanding the transition away from PVC and DEHP-containing medical devices
 - How can Green Chemistry aid this process?
 - Conclusions

Background:

Notable adverse trends in male reproductive health...

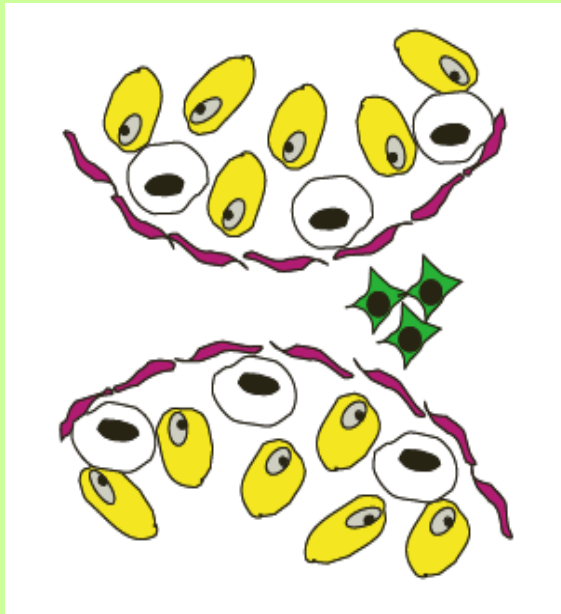
□ Testicular Dysgenesis Syndrome (Skakkebaek, 2001)

- Hypospadias
- Decreasing sperm counts
- Cryptorchidism
- Testicular abnormalities
- Epididymal abnormalities
- Testis cancer

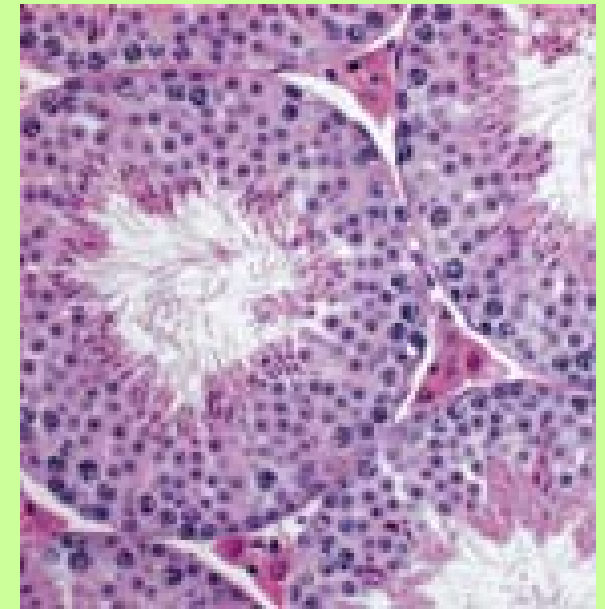
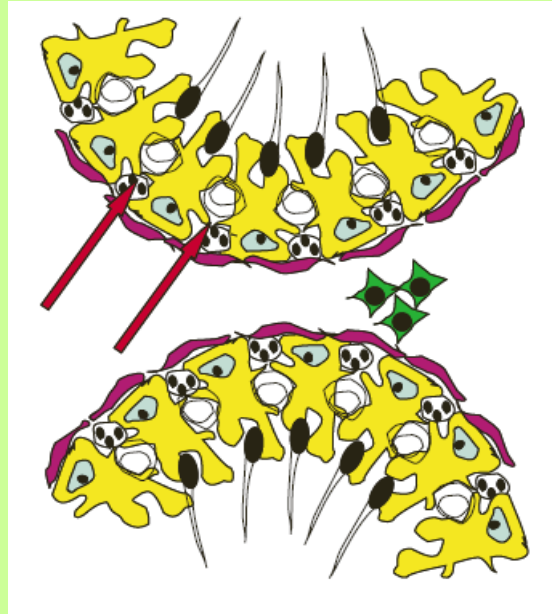


Background: Male reproductive Development

Immature Testis

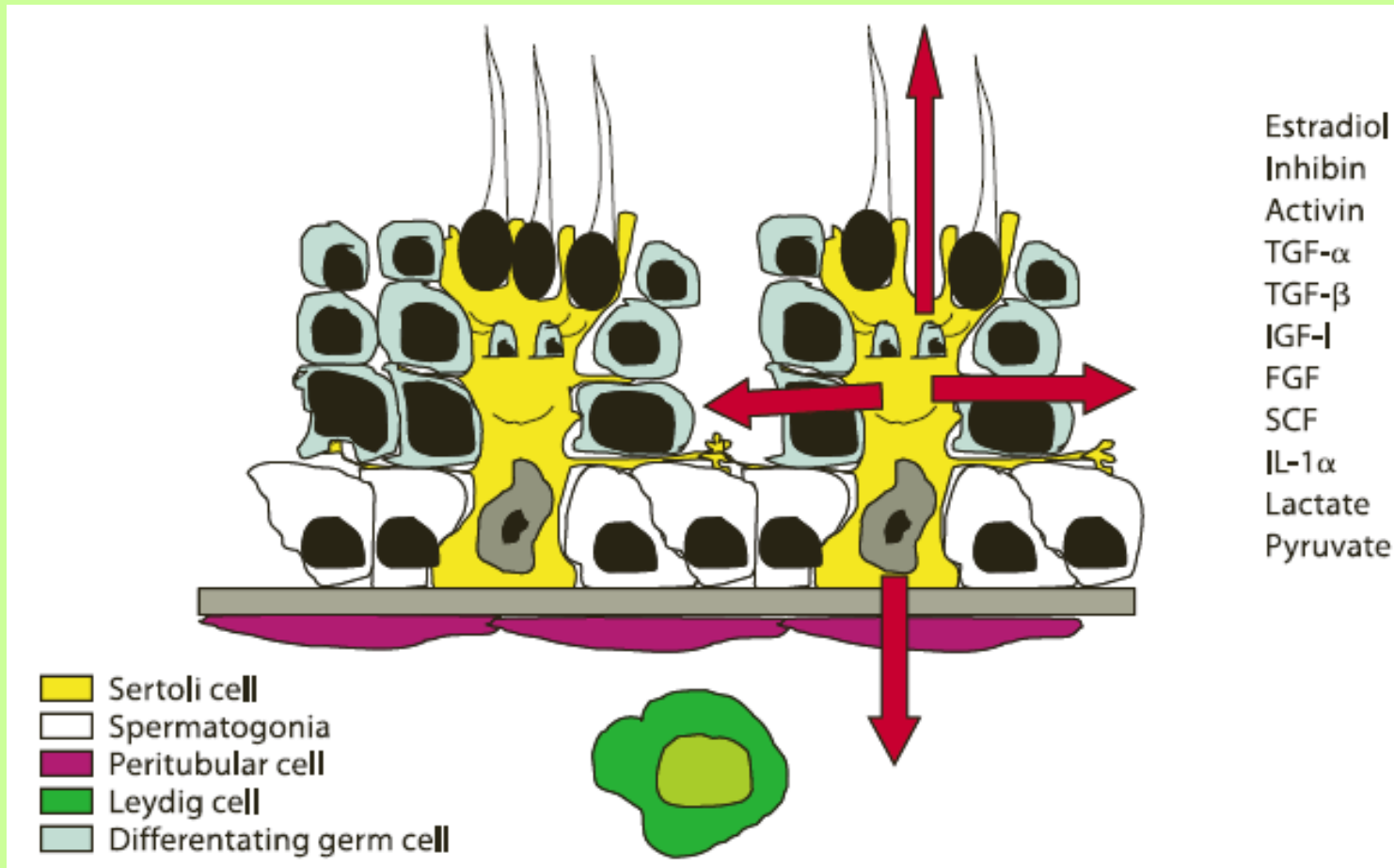


Mature Testis

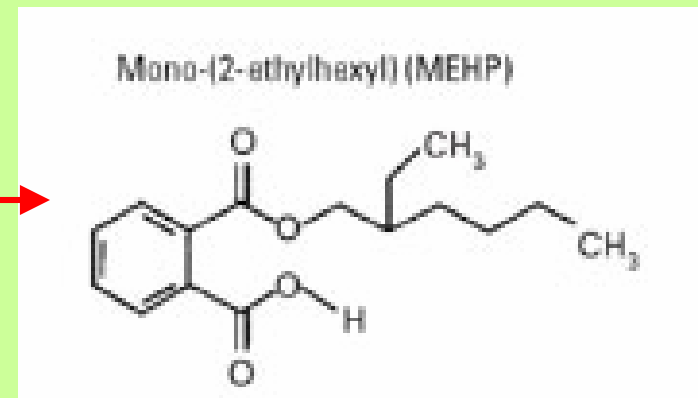
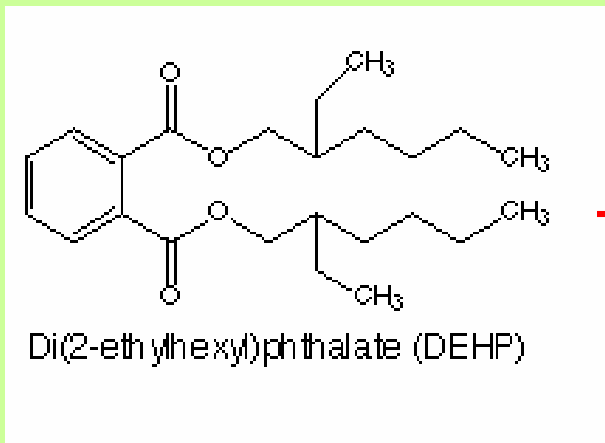


Background:

Sertoli cells are the nurse cells of the testis



Background: DEHP and MEHP



- ❑ Di-2-ethylhexyl phthalate (DEHP) is a low cost plasticizer used to make PVC flexible.
- ❑ DEHP exposure can occur in medical settings due to the heavy reliance on PVC for medical equipment.
- ❑ MEHP is the active metabolite of DEHP and is a known Sertoli-cell toxicant.

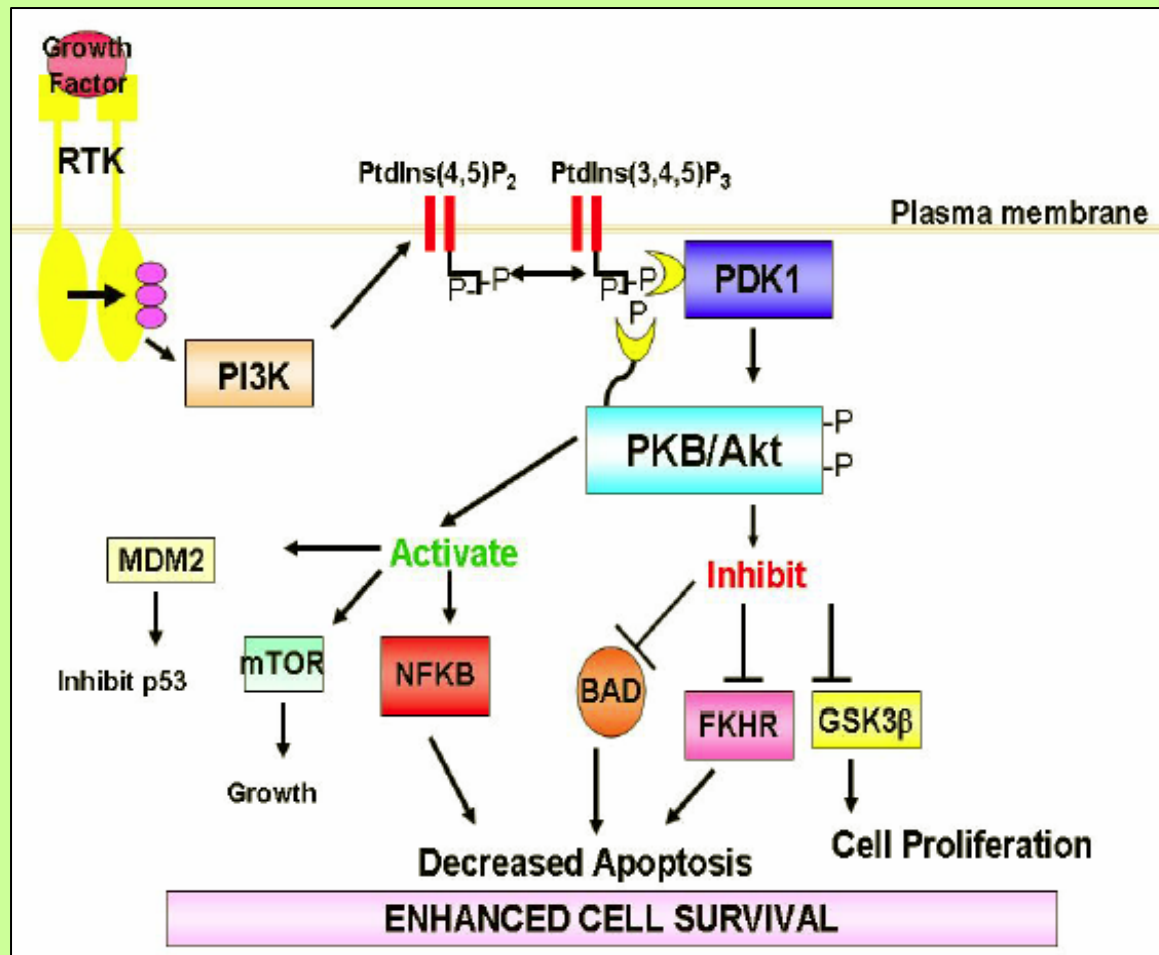
Background: MEHP and Pediatric Health

- Neonates face an increased risk from phthalates compared to adults
 - Developing metabolic pathways
 - Rapid growth and development
 - Small size = larger exposure dose

- Infants in medical settings (like the NICU) are exposed to physiologically relevant levels of phthalates through PVC medical devices.
 - Lehmann et al (2004)



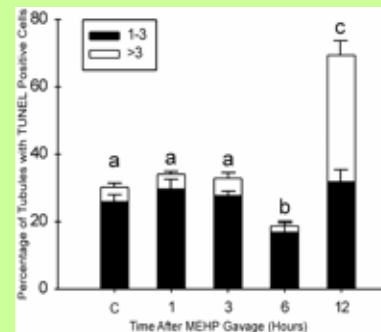
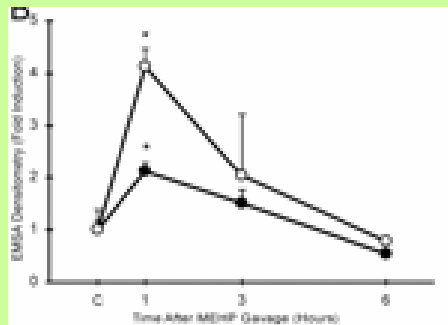
Background: The PI3K/Akt signalling pathway



Background:

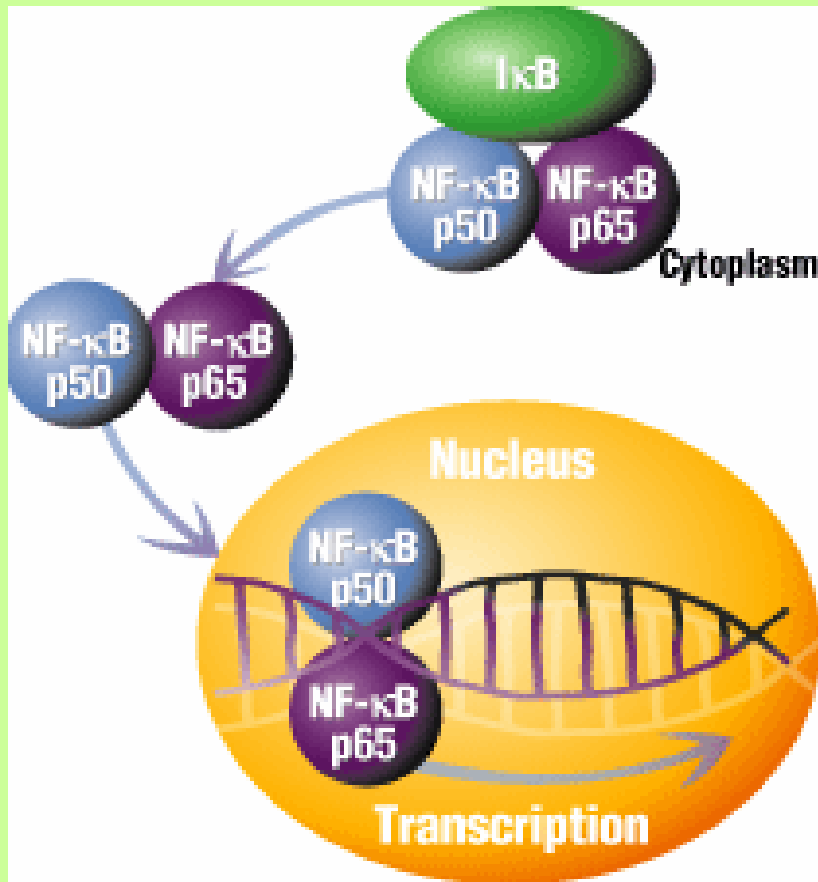
NF- κ B

- NF- κ B, a transcription factor studied primarily in the immune system, has been localized to spermatocytes and Sertoli cells.
 - Upregulates genes related to injury-induced apoptosis
- Rasoulpour et al (2004, BOR) found that, in the rat:
 - Incidence of apoptosis declines briefly following MEHP, while NF- κ B activity increases.



- It is thought that NF- κ B is involved in this immediate protective phenomenon.
- Oxidative stress has been shown to activate the NF- κ B signal transduction pathway

Background: NF- κ B pathway



- When NF- κ B is activated:
 - I κ Bs are phosphorylated
 - NF- κ B subunits are freed and move into the nucleus
 - NF- κ B subunits bind to DNA and trigger transcription.

Exposure Paradigm

**MEHP
exposure
(500 mg/kg
by gavage)**



0h



1h



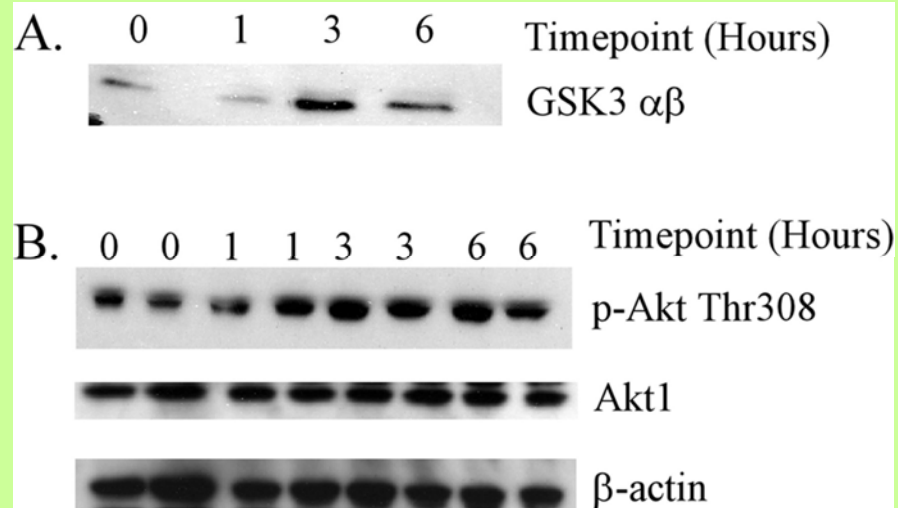
3h



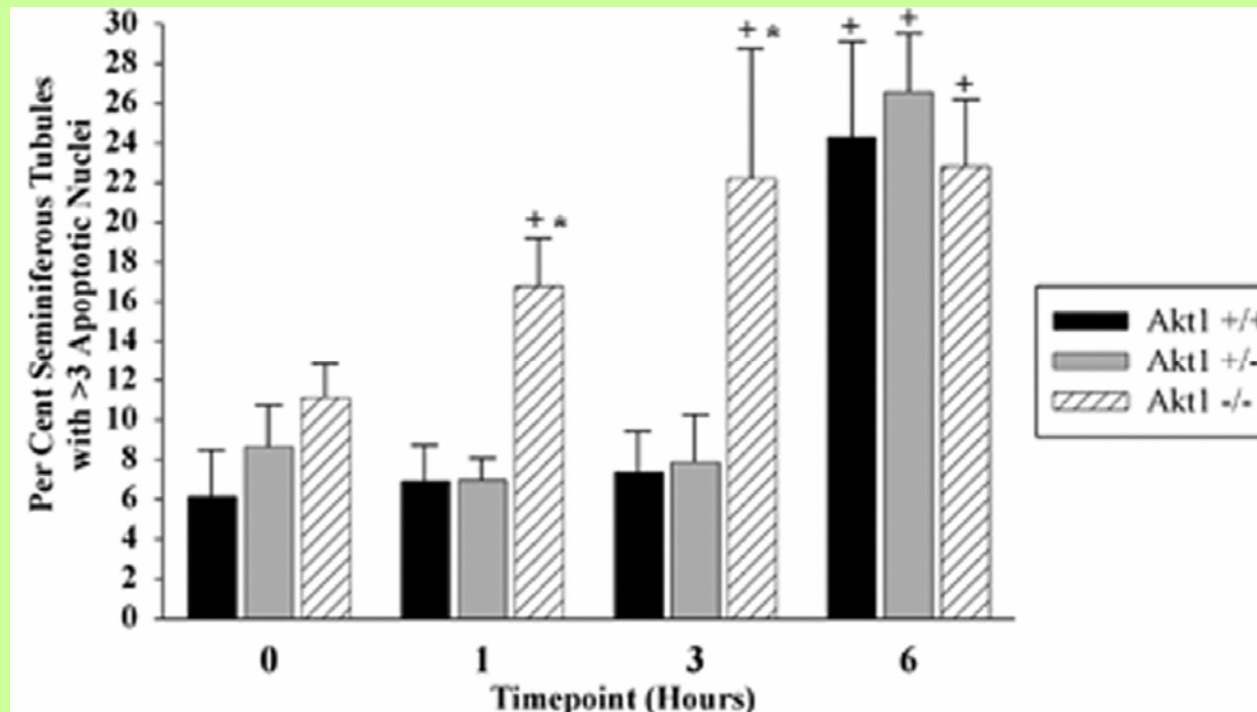
6h



The Akt signaling pathway is activated in response to exposure to 500 mg/kg MEHP



Akt1 suppresses MEHP-induced germ cell death.



Part 1: Specific Aims

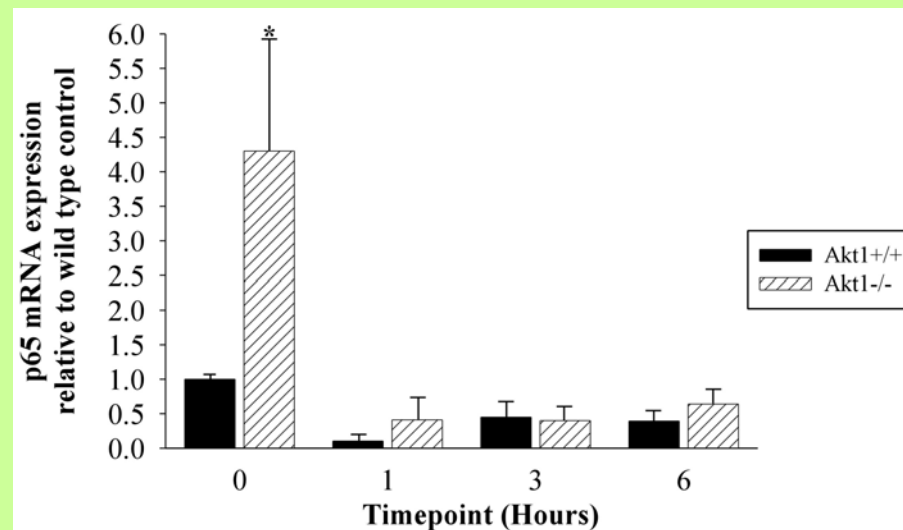
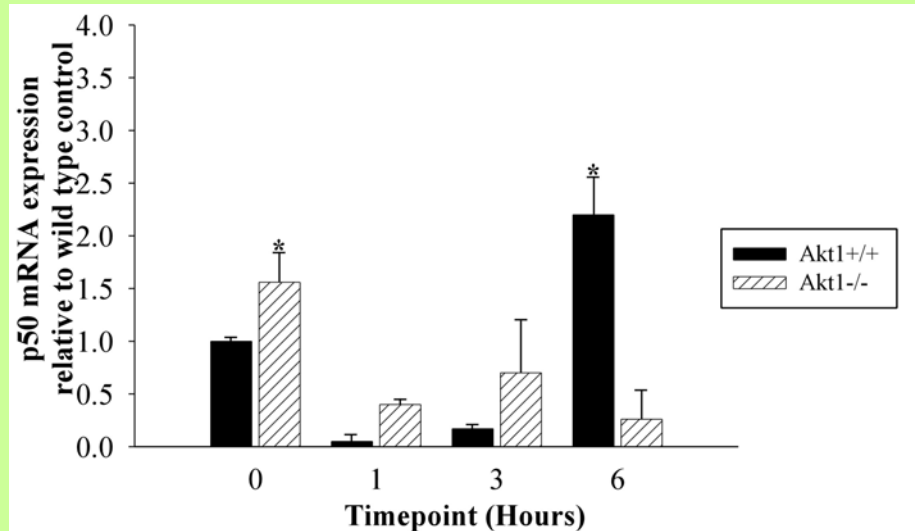
- By what mechanism does the Akt1 gene produce a protective effect in response to MEHP?
- Does NF- κ B activation play a role in the Akt1 cytoprotective response ?

Experimentation:

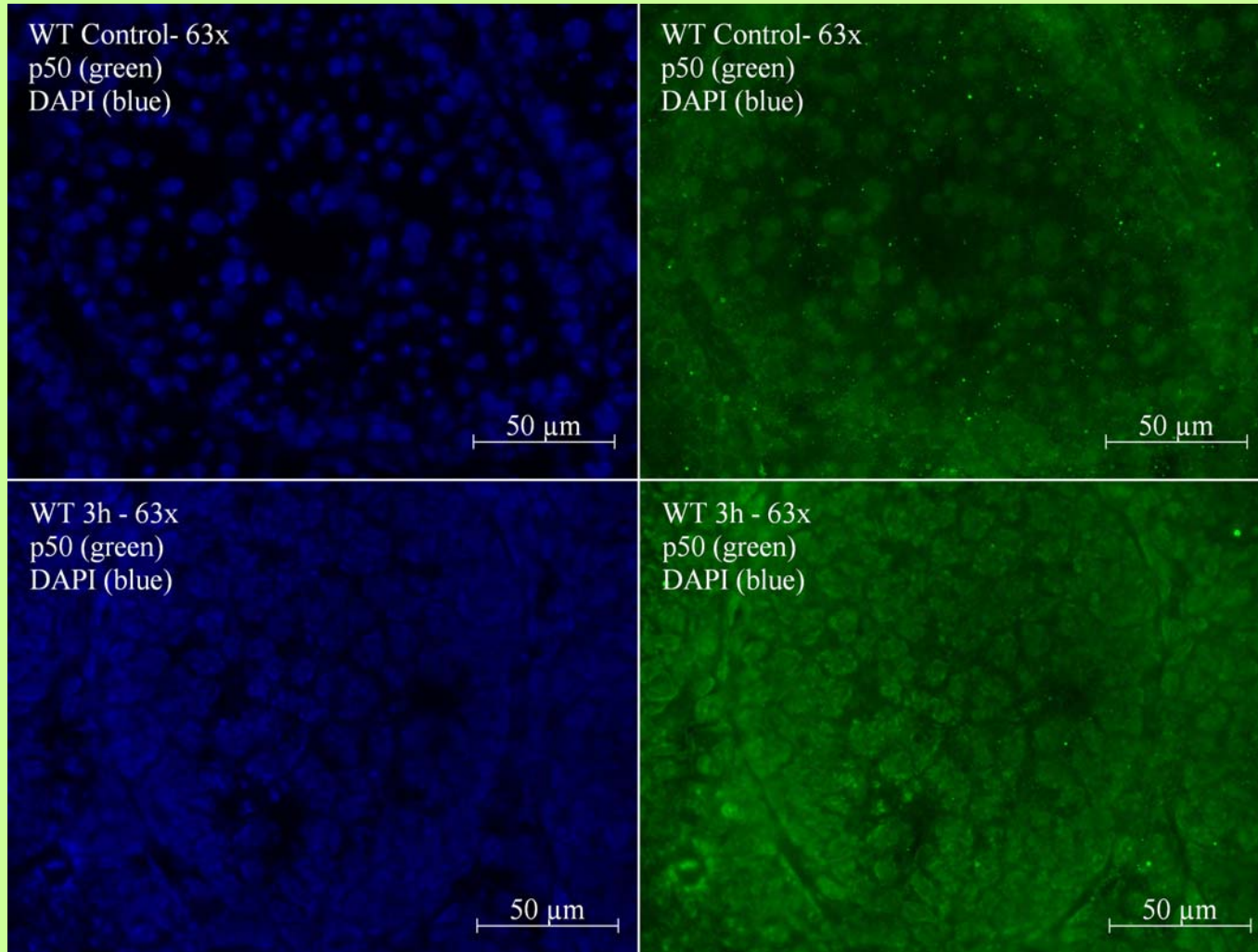
Assessment of NF- κ B activation and the consequences of Akt1 loss

- Localization and activation of subunits
 - p50
 - p65
 - **Immunohistochemistry**
 - ** RT-PCR**
- Phosphorylation of I κ Bs
 - **Western Blots**
- Expression of downstream targets of NF- κ B
 - **Western Blots**
 - **RT PCR**
- Stress responses induced by loss of Akt1
 - **Affymetrix**
 - **RT-PCR**

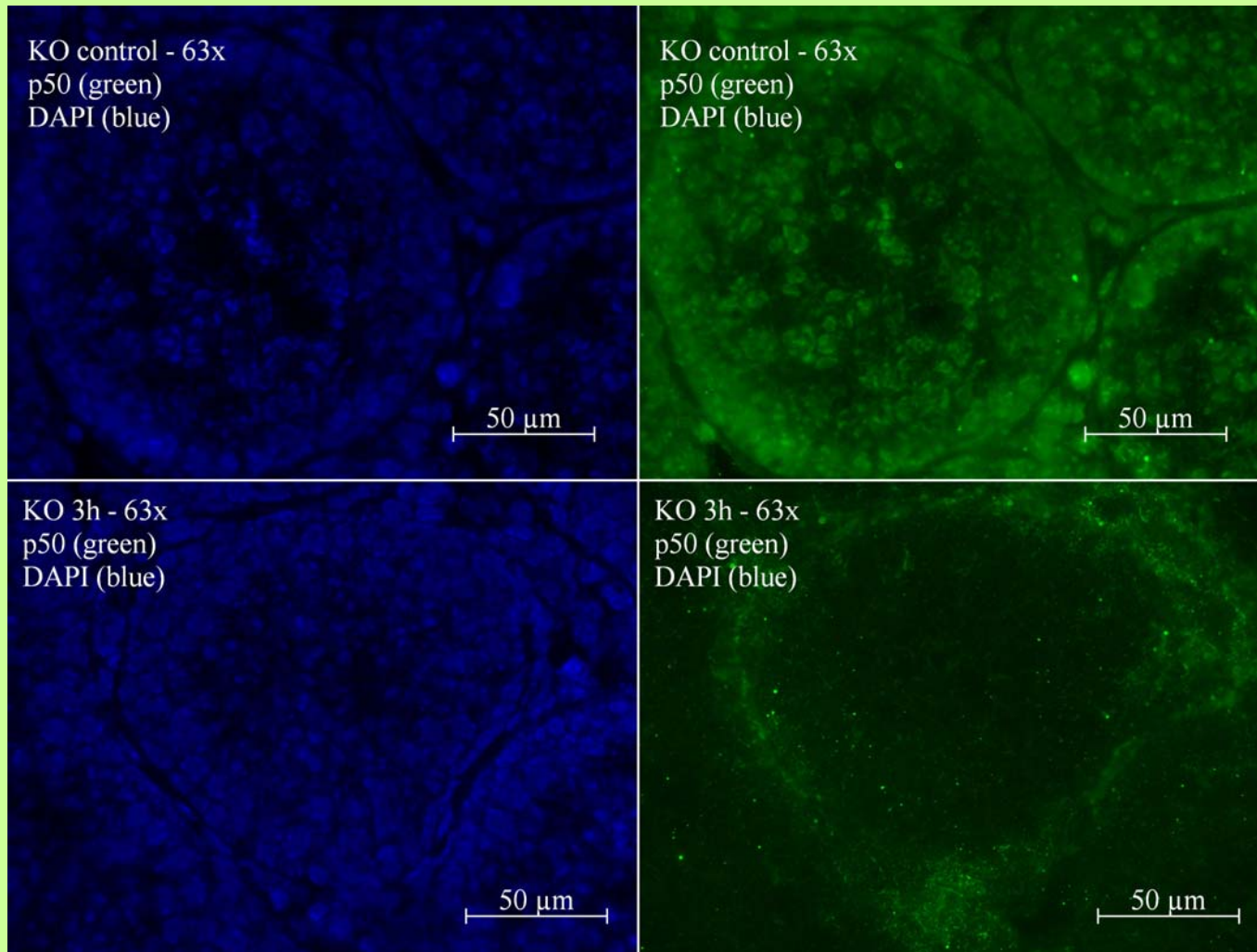
P50 and p65 are significantly elevated in Akt1-deficient control mice



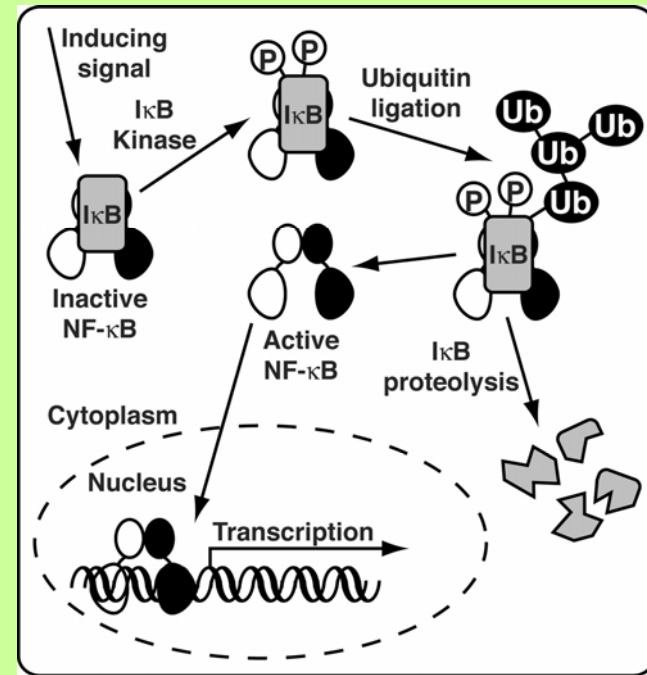
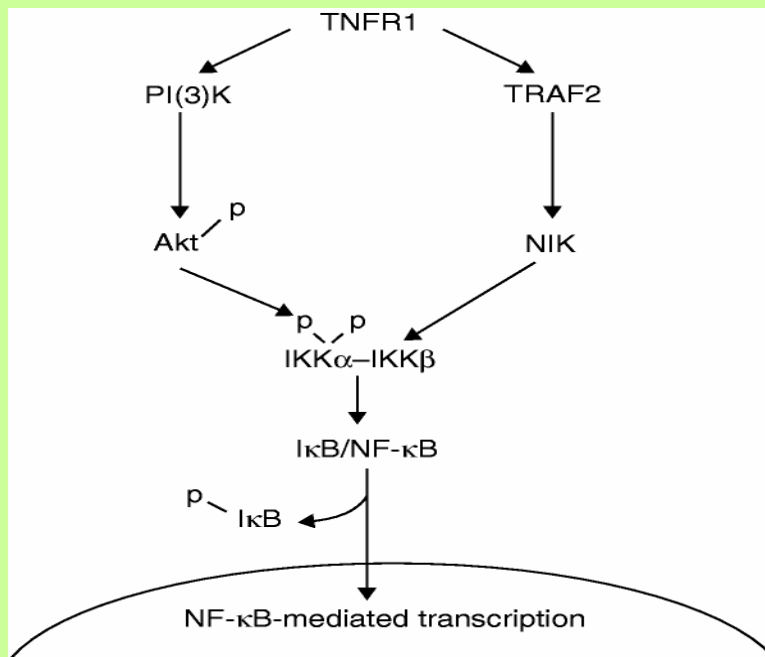
NF- κ B subunit p50 localizes to the nucleus in Akt1-wild type seminiferous tubules



p50 localizes to the nucleus in Akt1-deficient mice at the control time point, not at three hours



Phosphorylation of I κ B

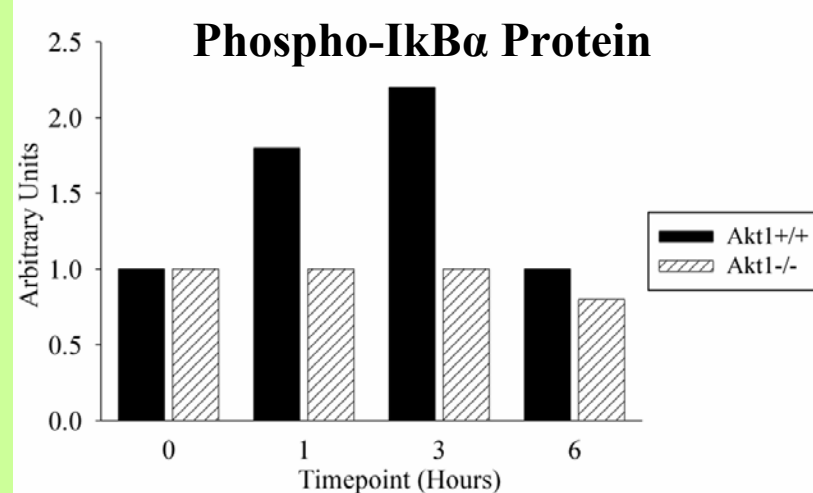
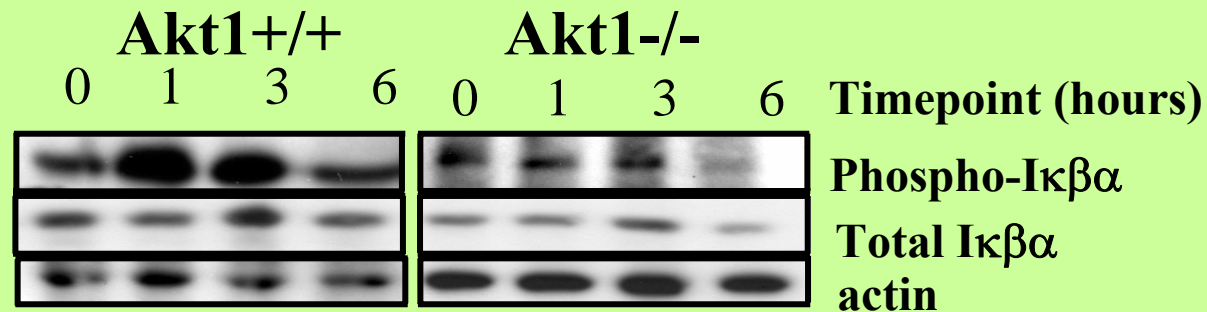


Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, and Donner DB. Nature 401, 82-85(2 September 1999)

http://www.chemistry.sdsu.edu/graphics/huxford_nfkb_activation.jpg

- ❑ Akt phosphorylates the I κ B complex
- ❑ I κ B must be phosphorylated in order for NF- κ B activation
- ❑ Leads to cell survival.

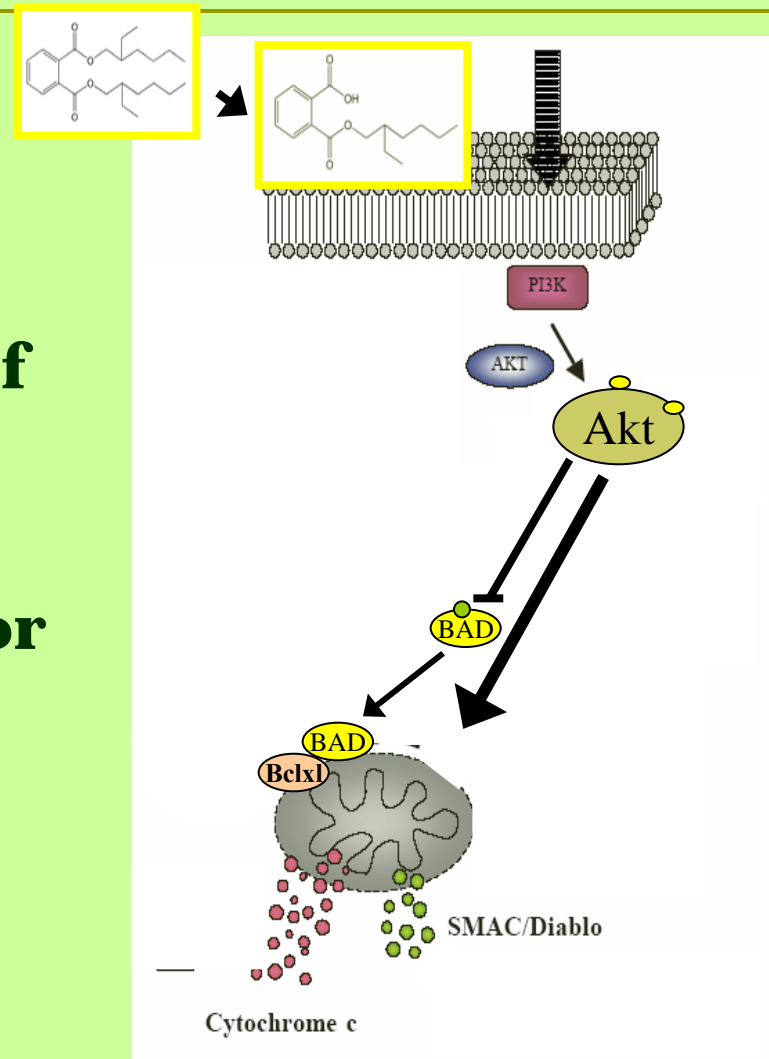
MEHP exposure leads to phosphorylation of I κ B α and is dependent on Akt1 status



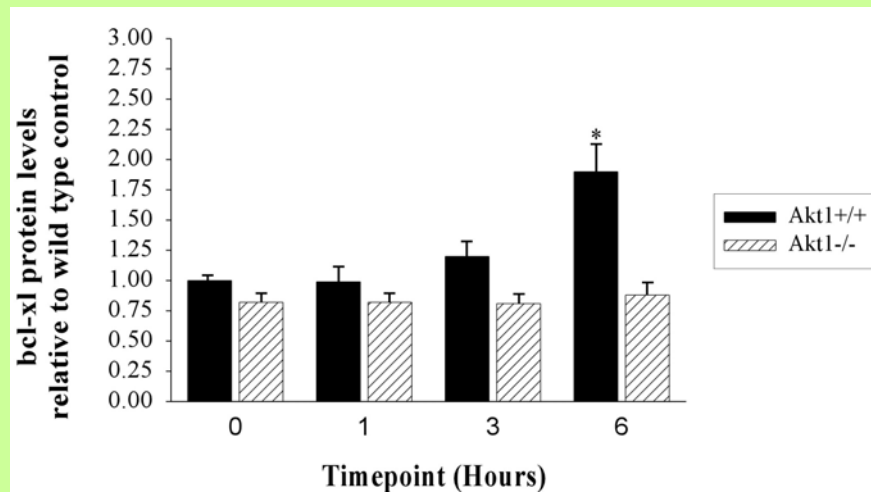
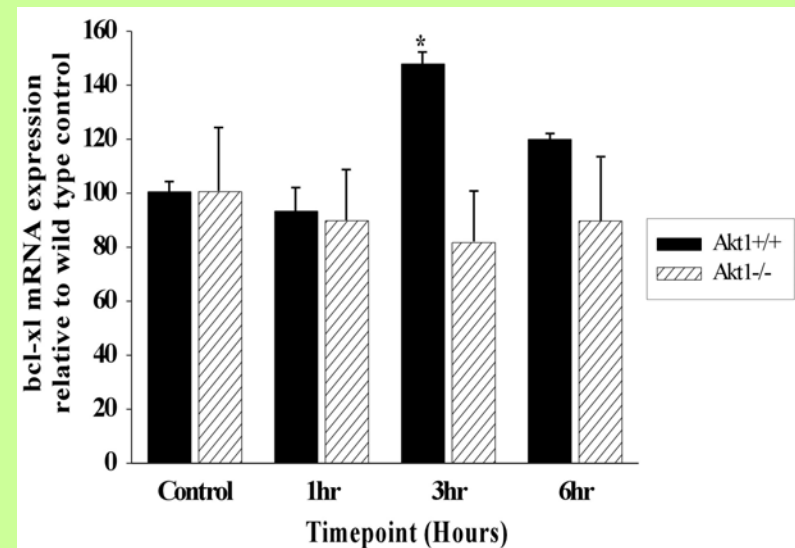
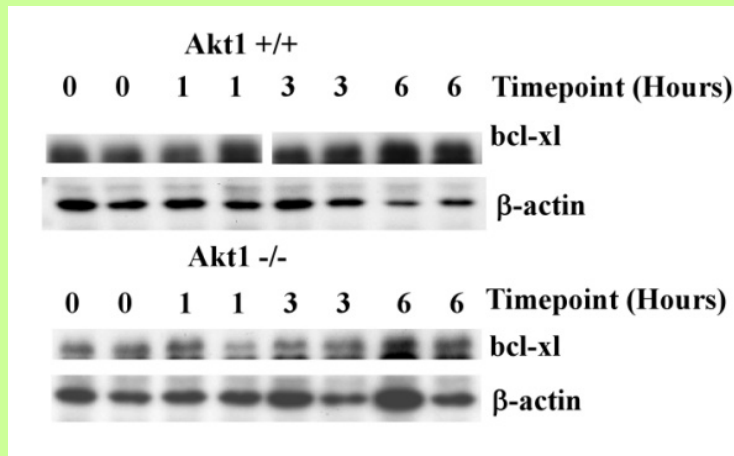
Downstream Targets of NF- κ B/Akt

□ Bcl-Xl

- Member of the Bcl-2 family
- Mediates the release of cytochrome c
- Cell survival protein
- Antiapoptotic regulator



Akt1-deficient mice exhibit reduced protein and mRNA expression of Bcl-Xl



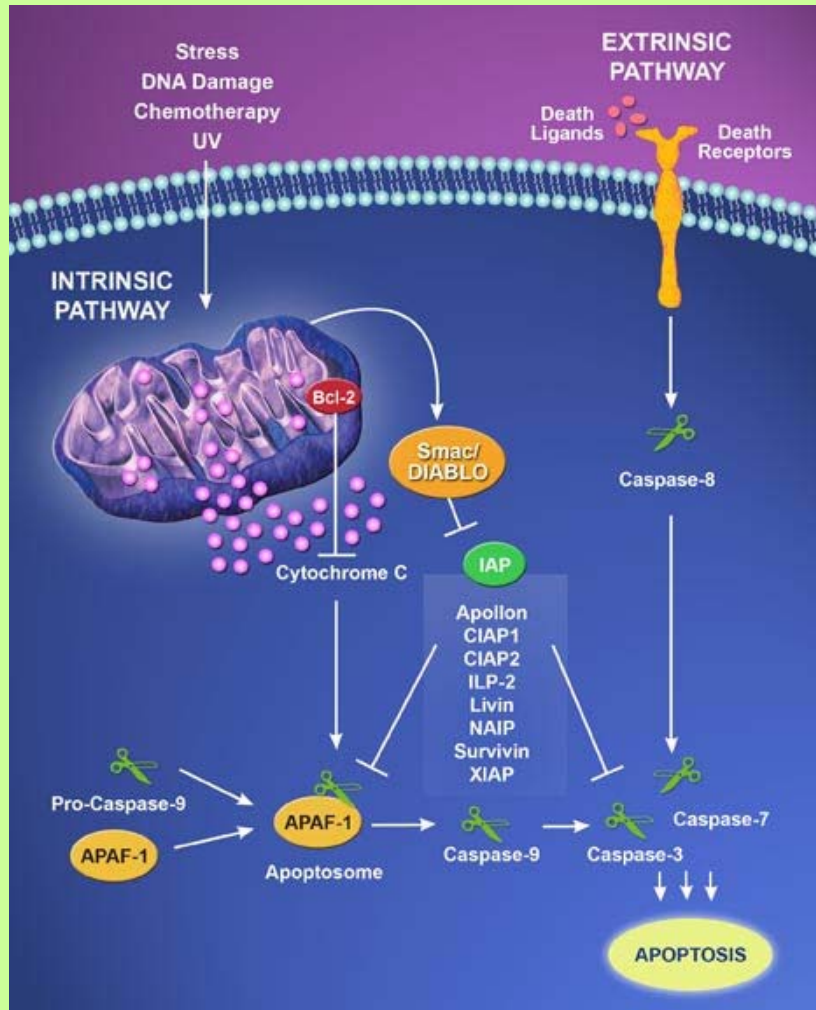
Experimentation: Gene Expression Study

locusID	symbol	WT.tr	KO.tr	Diff	P1	P2
243302	LOC243302	1.62	-1.41	-3.03	0.000151	0.000529
15430	Hoxd10	-0.349	0.0373	0.386	0.000183	9.68e-05
73473	Iws1	0.188	-0.0394	-0.227	0.00129	0.00133
18430	Oxtr	-0.896	-0.00996	0.886	0.00135	0.000381
66593	Diablo	0.342	-0.144	-0.486	0.00141	0.00288
52150	Kenk6	0.84	0.238	-0.602	0.00151	2.57e-05
270166	Clpx	0.532	-0.185	-0.717	0.00166	0.00283
71891	Cdade1	-0.523	0.076	0.599	0.00221	0.00175
28109	D10Wsu102e	0.528	0.0603	-0.468	0.00227	0.000292
22695	Zfp36	0.748	0.115	-0.632	0.00238	0.000208
116940	Ncoa6ip	0.573	-0.0235	-0.596	0.00272	0.00122
28001	D16Wsu65e	0.389	-0.13	-0.519	0.00282	0.00468
73625	1810008I18Rik	-0.208	-0.0138	0.195	0.00351	0.000755
319584	9330107J05Rik	0.434	-0.0927	-0.526	0.00403	0.00454
216850	Jmjd3	0.494	0.0407	-0.453	0.00403	0.000778
235293	Sc5d	-0.175	0.0514	0.227	0.00427	0.00637
68067	3010026O09Rik	0.251	-0.0623	-0.314	0.00431	0.00554
27359	Sytl4	0.364	-0.115	-0.479	0.00434	0.00691
67420	Mlstd2	-0.222	0.0429	0.265	0.00459	0.00482
12153	Bmp1	-0.4	0.00799	0.408	0.00463	0.00197
227325	Dner	-0.362	0.00865	0.37	0.00466	0.00203
434778	6330534C20Rik	0.288	-0.042	-0.33	0.00514	0.00445
229681	St71	-0.245	0.0739	0.319	0.00521	0.00804

- To further identify downstream targets activated by Akt1 and MEHP exposure:
 - Gene expression studies:
 - Akt1 – wild type control, n=3
 - Akt1 – wild type 3 hour, n=3
 - Akt1 – deficient control, n=3
 - Akt1 – deficient 3 hour, n=3

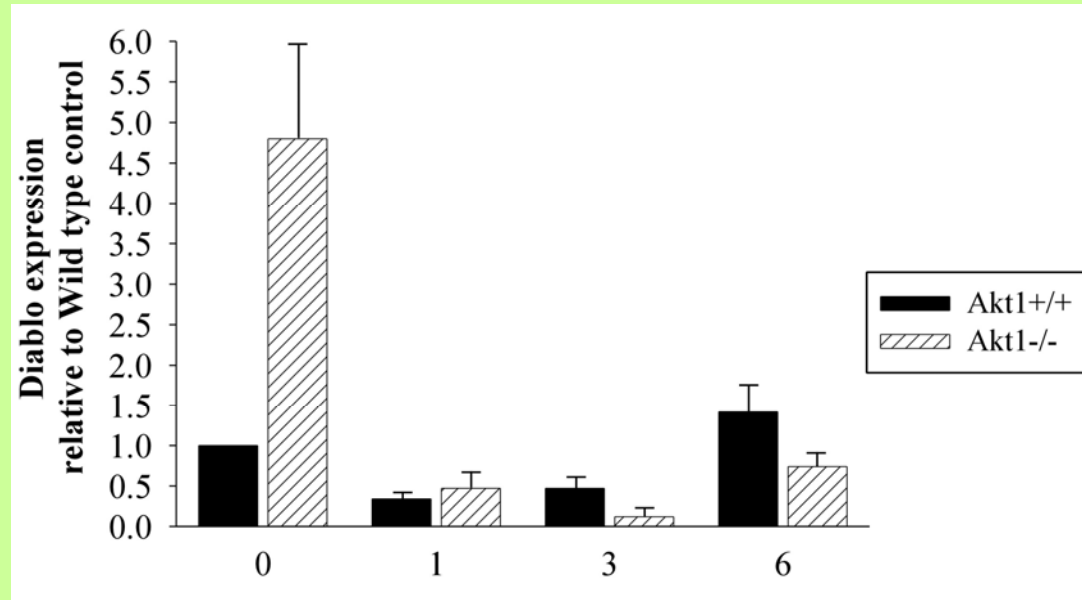
- SMAC/Diablo

SMAC/Diablo



- The mitochondrion integrates cell death pathways
- SMAC/Diablo:
 - Neutralizes the inhibitor of apoptosis proteins
 - Disruption is a hallmark of early apoptosis
 - implicated in the release of cytochrome c
 - in part regulated by continuous oxidative stress in the mitochondria

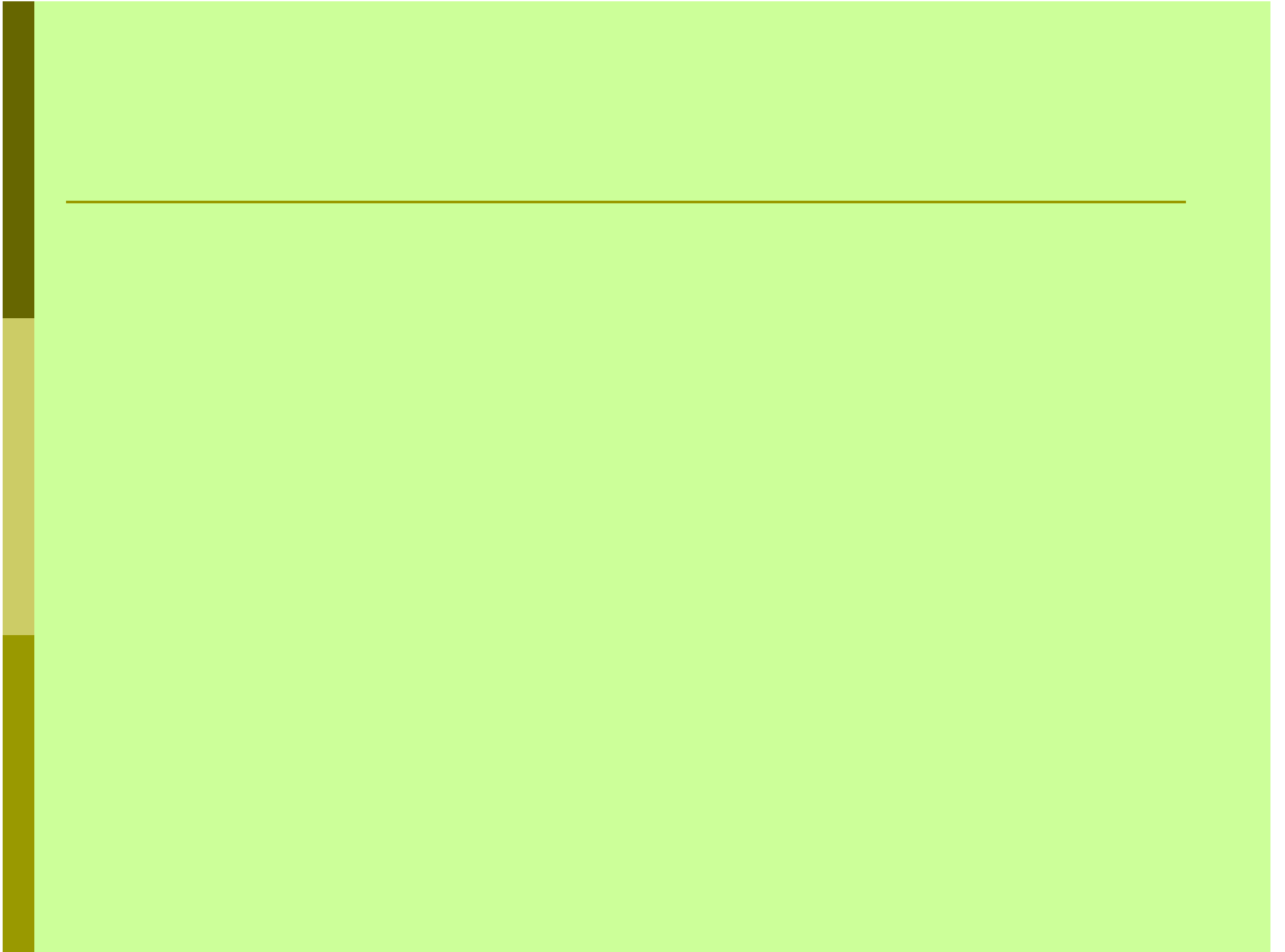
SMAC/Diablo expression is significantly elevated in Akt1-deficient controls



- MEHP exposure causes a significant decrease in expression.
- Elevated SMAC/Diablo expression indicate a possible stress response due to loss of Akt1
- Supports the conclusion that Akt1-deficient mice are more sensitive to MEHP exposure

Summary

1. Akt1 suppresses MEHP-induced germ cell apoptosis
2. Akt1-deficient control mice have elevated p50 and p65 mRNA expression which suggests increased NF-kB activation.
3. Bcl-Xl expression is induced in Akt1-wild type testes.
4. Data suggests a role for mitochondrial injury following MEHP exposure, and that this response is exacerbated upon loss of Akt1.



Part II: Medical Responses to Phthalate Exposure

What's the big picture?



Background: Green Healthcare

□ Definition:

- The incorporation of environmentally friendly practices in medical settings in order to facilitate environmental and public health
- Healthcare practitioners have an obligation to eliminate and avoid practices that are unhealthy for people and the environment

□ The Precautionary Principle:

- Shifts the burden of proof to those who advocate using a chemical rather than those who advocate precautionary measures.
- Emphasizes planning, safety assessment, and anticipatory action
- Advocates preventative measures aimed at avoiding environmental disaster even in the absence of conclusive scientific evidence.

Background:

Statements from Regulatory Agencies

- ❑ **FDA Public Health Notification: PVC Devices Containing the Plasticizer DEHP**
 - Some individuals can be exposed to high levels of DEHP through certain medical procedures
 - High-risk procedures:
 - ❑ Exchange transfusion
 - ❑ ECMO
 - ❑ Total Parenteral Nutrition
 - ❑ Hemodialysis and massive infusions
 - The risk posed by exposure to DEHP is dependent on the patient's sensitivity and the received dose.
 - ❑ The male fetus, male neonate, and peri-pubertal male are high-risk
 - ❑ Dose is largely dependent on the frequency and duration of the procedure.
- ❑ **These findings are echoed by the ATSDR and the AMA.**

Experimentation: Understanding the Transition

- **What characteristics make a hospital more likely to phase phthalates out?**
 - Size
 - Age
 - University affiliation
 - Membership in major healthcare system
- **What steps must a hospital take to begin this transition?**
 - Who are the key players?
 - What organizations are involved?
 - Where do you start?

Experimentation: Methods

□ **Study Population**

- Hospitals identified as the ‘Top 25 Pediatric Hospitals of 2008’ by US News and World Report
- Hospitals that participated in Health Care Without Harm’s NICU No Harm online survey
- Hospitals that have signed Health Care Without Harm’s NICU pledge
- NICU pledge analysis: $n = 107$
- Hospital Survey: $n = 16$

Experimentation: Methods

□ **The Survey**

■ 18 questions

- **Are you aware of the Food and Drug Administration (FDA) DEHP Public Health Notification?**
- **Do you have a purchasing policy that prefers non-DEHP and/or non-PVC products?**
- **Have you eliminated the use of any PVC or DEHP-containing devices?**
- **If your hospital has taken action to remove DEHP, what motivated this action?**
 - **FDA advisory?**
 - **Internal awareness?**
 - **External Education?**
 - **Other?**
- **If your hospital has taken action to remove DEHP, what challenges did you face?**

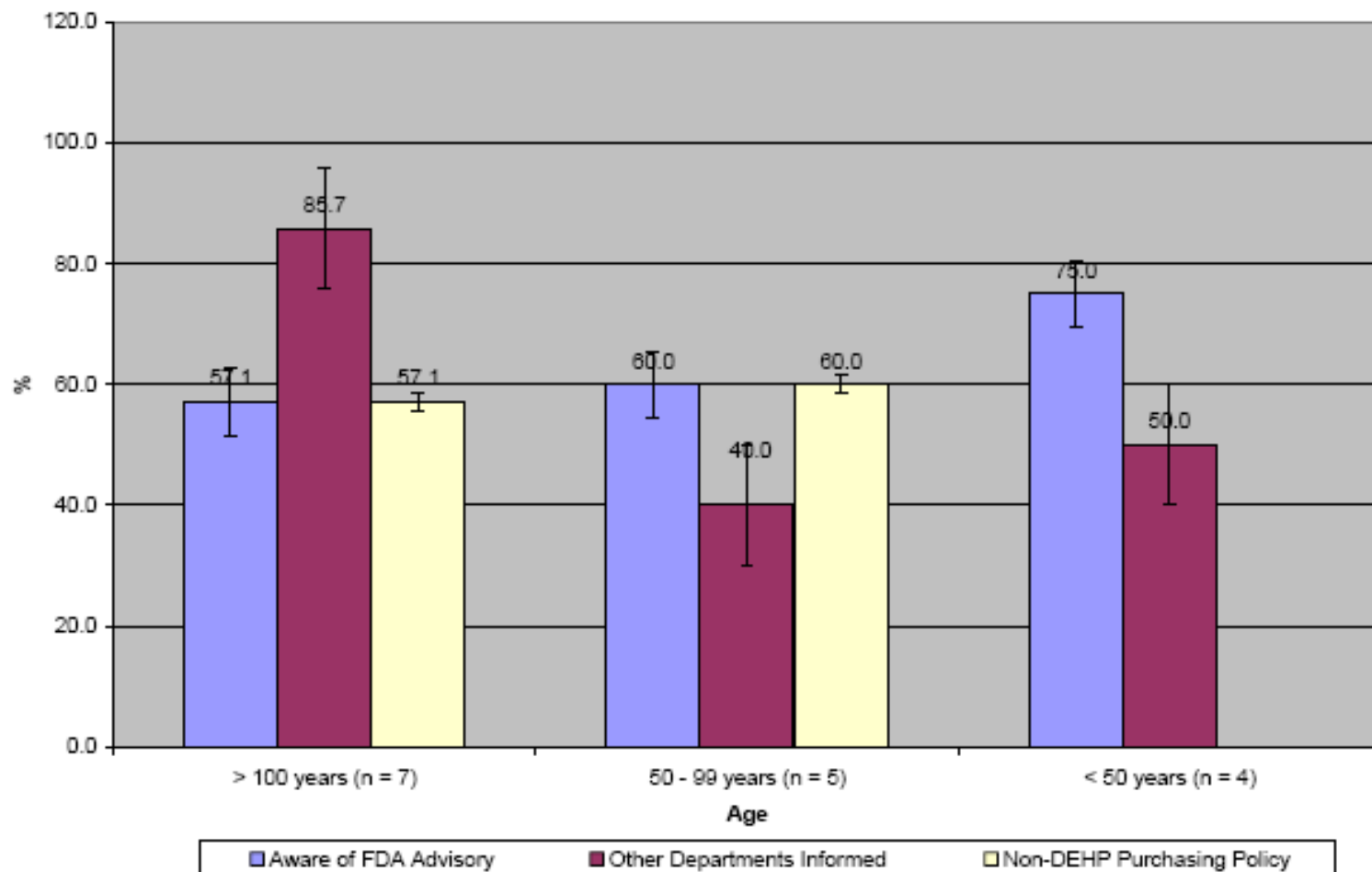
Experimentation: Methods

□ **Definitions**

- Age:
 - Time since reported foundation date
- Population:
 - Population of the city the hospital is located based on 2006 US Census data
- Region:
 - North East, South East, Mid West, or West
- Major Healthcare System:
 - Health maintenance organization or hospital management system that oversees the operations of hospitals in more than one state
- University Affiliation:
 - Major: teaching hospital or hospitals founded as a result of a university charter
 - Limited: hospitals that house biomedical researchers from neighboring universities
 - None

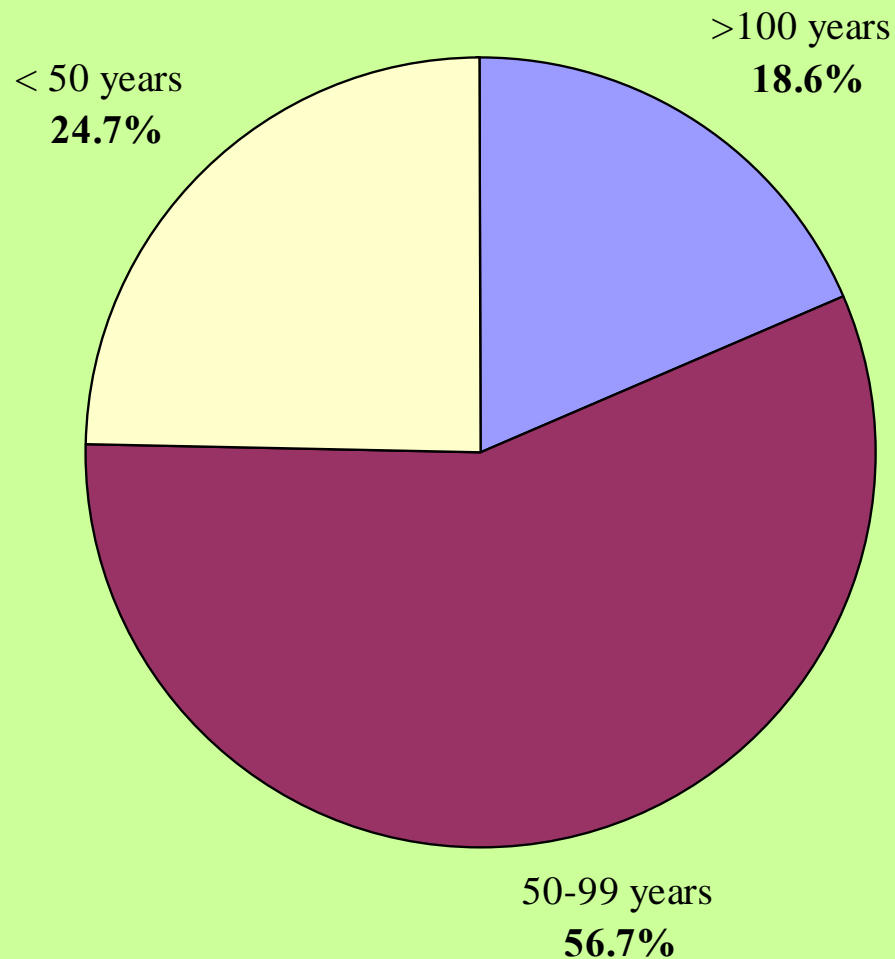
Experimentation: Age of Hospital

Influence of age on awareness of FDA advisory on DEHP, interhospital knowledge sharing, and presence of non-DEHP purchasing policy



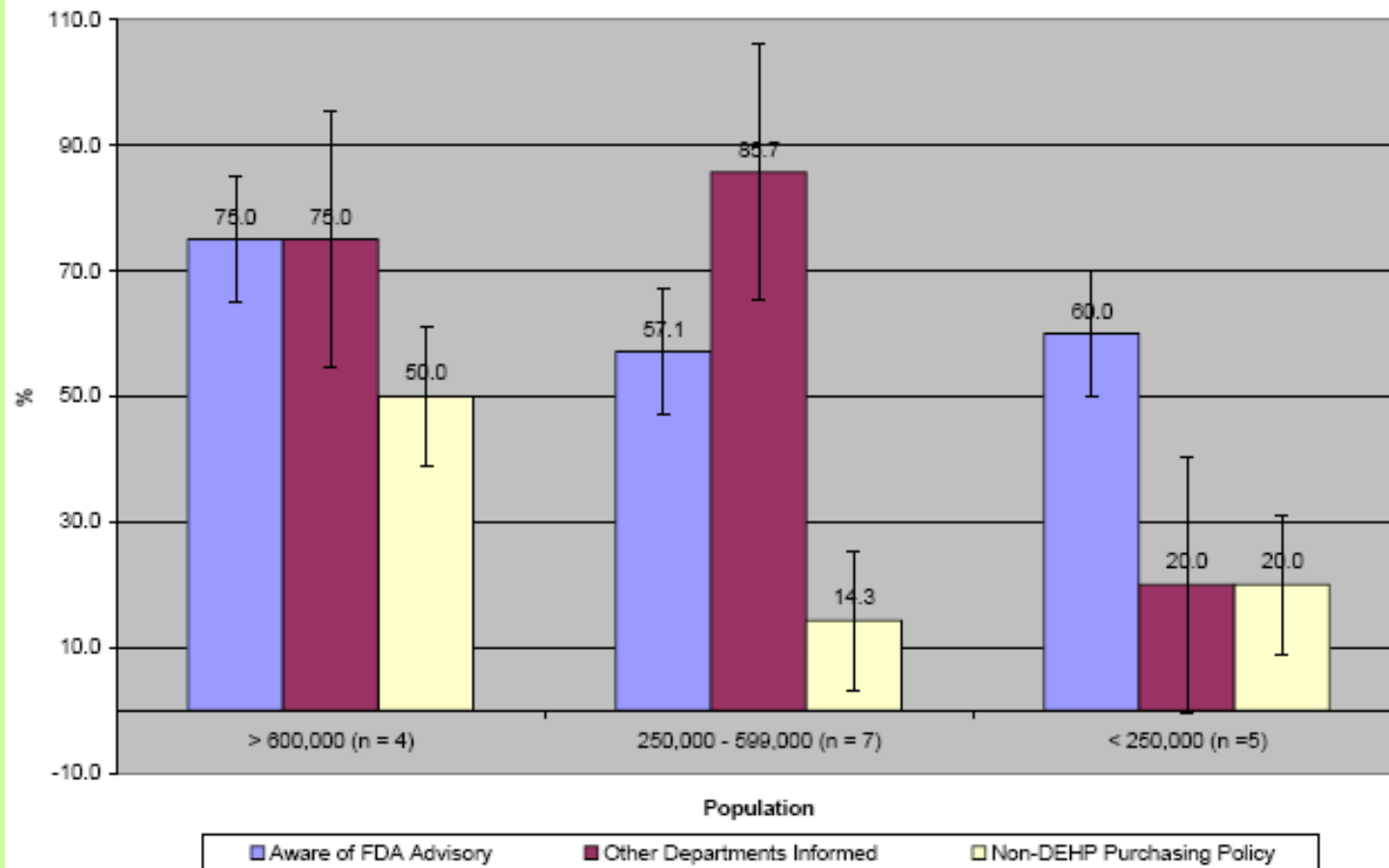
Experimentation: Age of Hospital

Age Distribution of NICU Pledge Hospitals



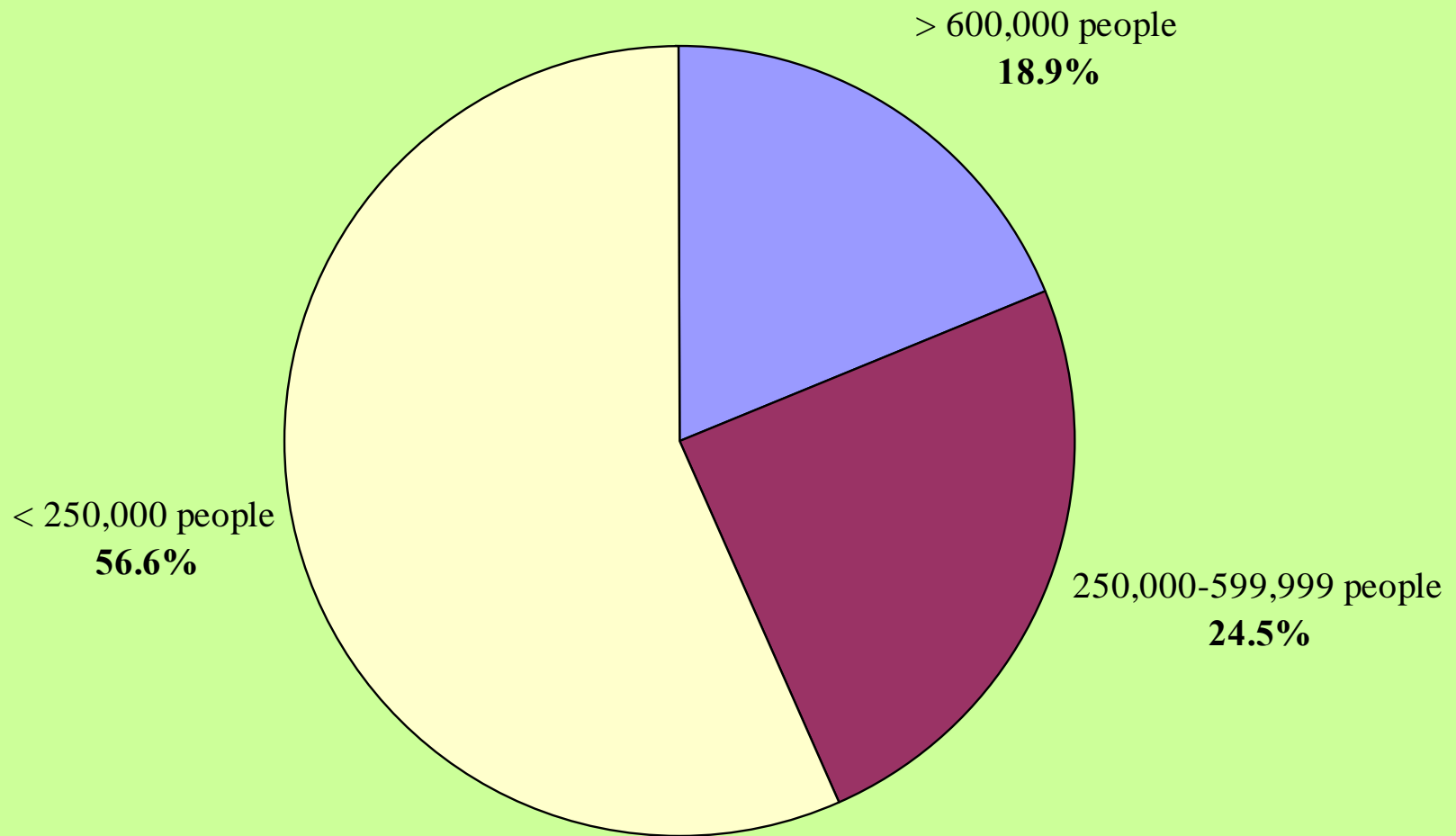
Experimentation: Population

Influence of population on awareness of FDA advisory on DEHP, interhospital knowledge sharing, and presence of non-DEHP purchasing policy

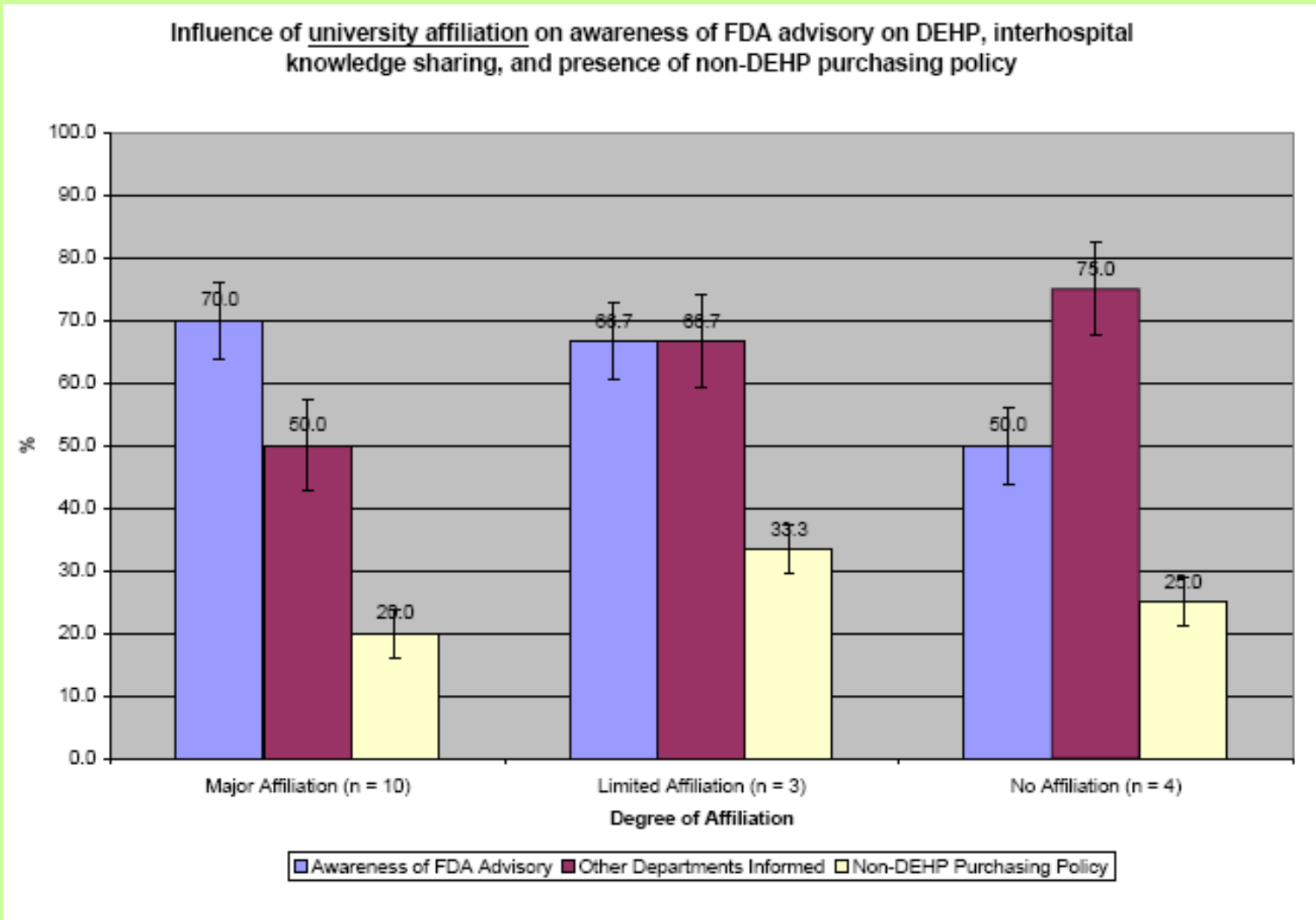


Experimentation: Population

Population Distribution of NICU Pledge Hospitals

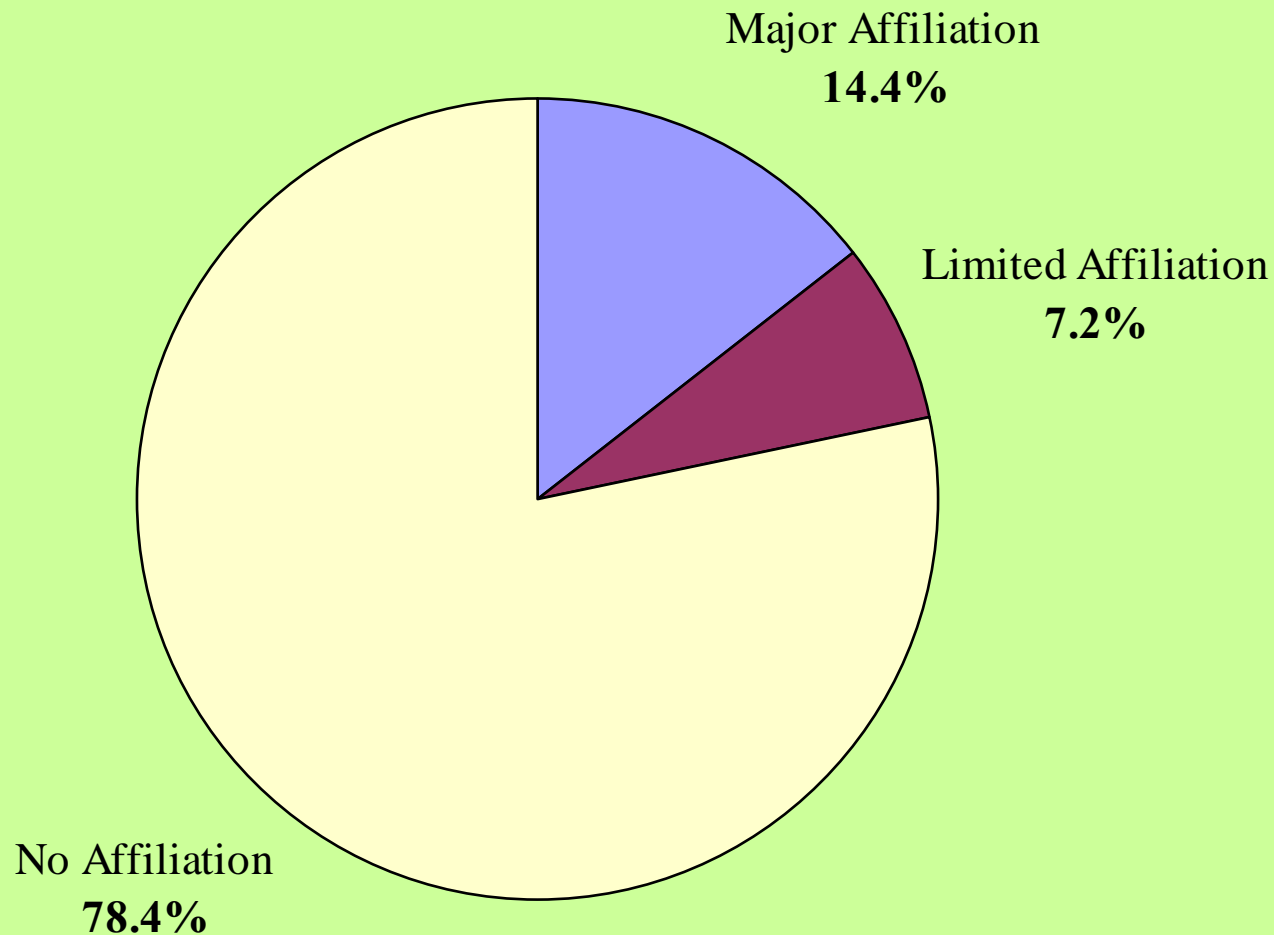


Experimentation: University Affiliation

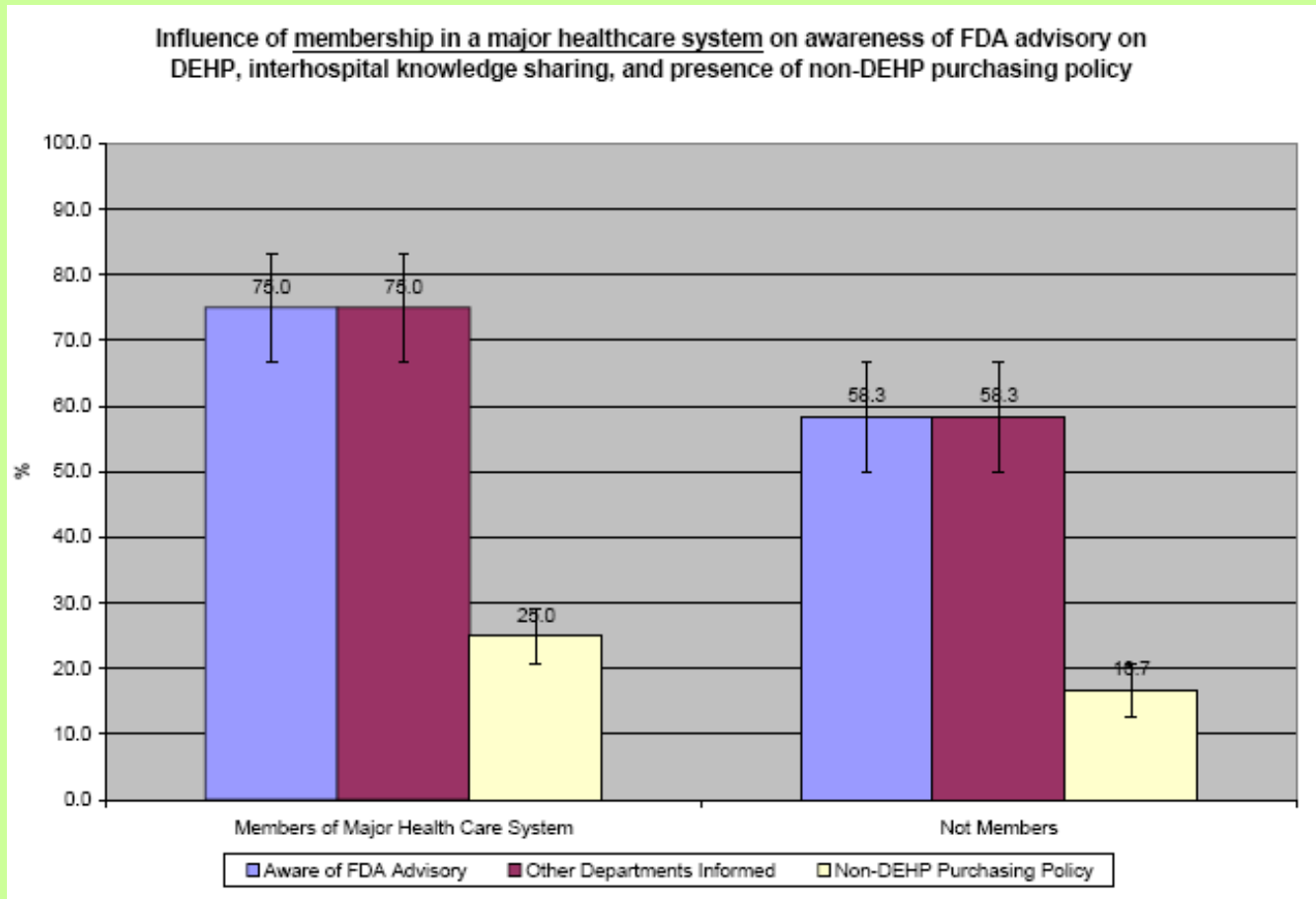


Experimentation: University Affiliation

Degree of University Affiliation Amongst NICU Pledge Hospitals



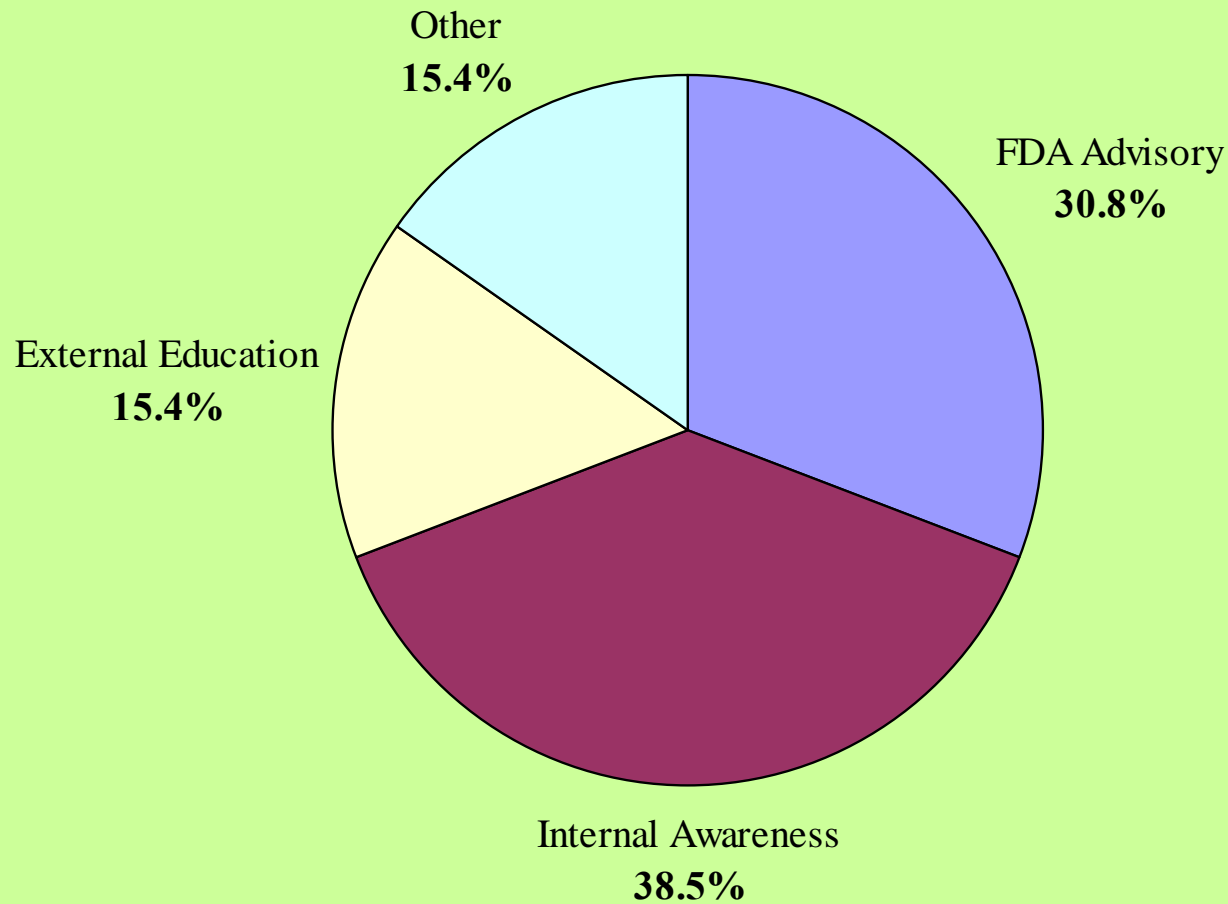
Experimentation: Membership in a Major Health Care System



- **59.8% of NICU pledge hospitals are members of major health care systems.**

Experimentation: Motivation

Motivation to Transition Away from PVC Plastic and DEHP-Containing Medical Devices



Experimentation:

Conclusions

1. Age and university affiliation do not have a significant impact on hospital behavior regarding DEHP.
2. Population may be a moderate predictor of hospital behavior
3. Membership in a major health care system is the strongest predictor of a hospital's DEHP initiatives
4. Hospitals are most motivated by statements from regulatory agencies and internal awareness

Experimentation: Limitations of the Study

- ❑ Study population is small
- ❑ Potential confounding
 - Age?
 - Population?
 - Location?
 - University Affiliation?
 - Membership in Major Health Care System?
- ❑ A good starting place...

Case Study:

RI Women and Infants' Hospital

□ **Basic Characteristics:**

- Age: 123 years
- Population: 175,255
- University Affiliation: Major
- Membership in Major Health Care System: Yes

□ **RI Women's and Infants Hospital's NICU is 'phthalate free.'**

- Began transition in February 2004
- Switch was motivated by an article recommended by a parent.

□ **Vendors were very responsive and provided 'phthalate free' devices very quickly.**

□ **Difficulties of transition:**

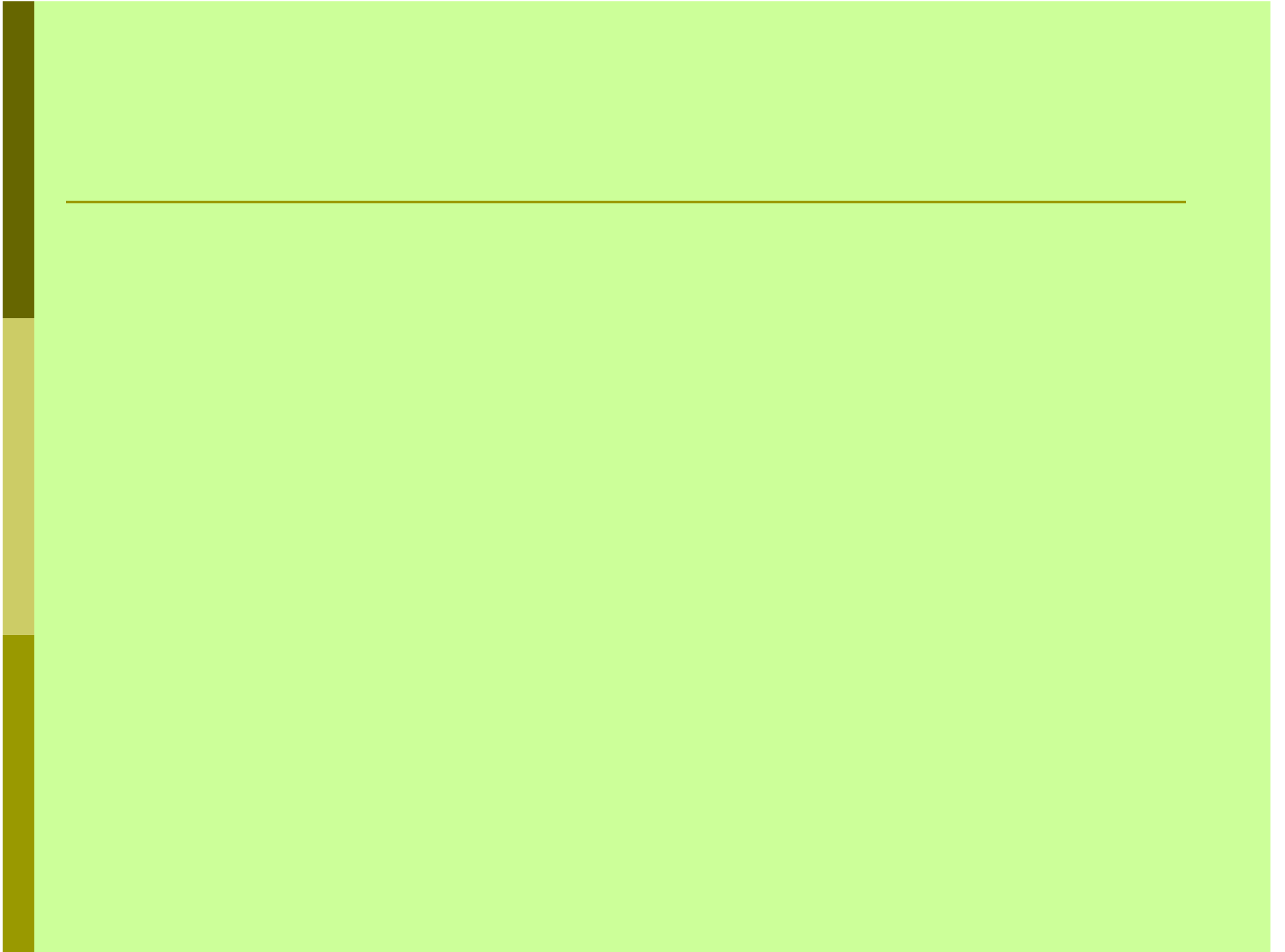
- Alternative devices are more expensive.
- Details of materials can be confusing to purchasing department.
- Nurses stated that new materials were difficult to use.

□ **Turnover took approximately 2 months to complete**

- Would have been faster but the purchasing department was "slow"

Thanks!

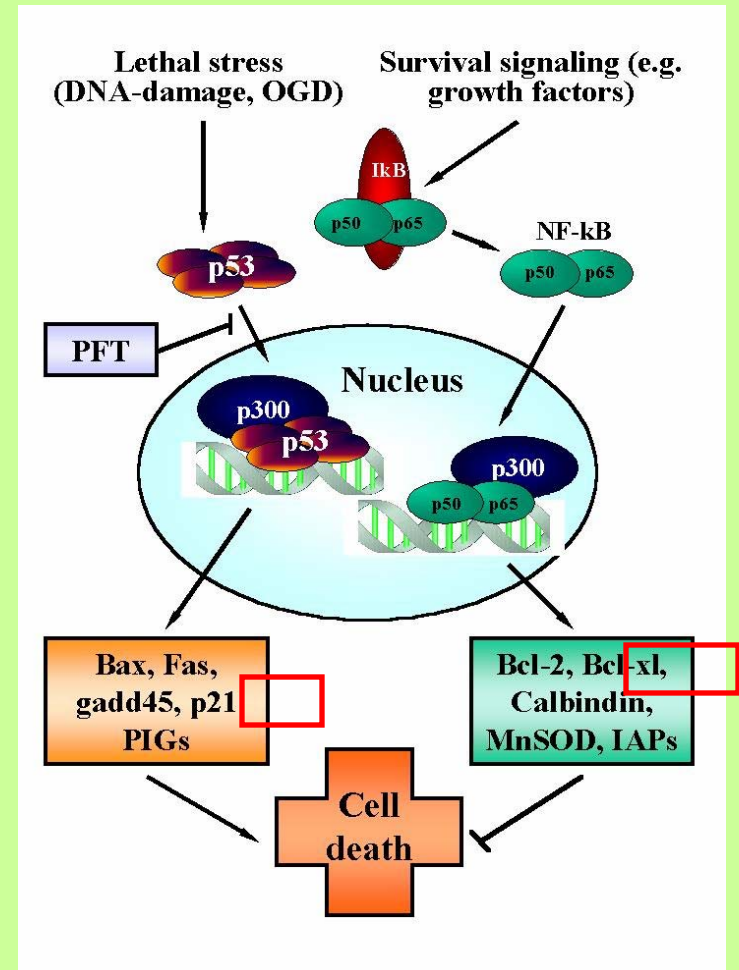
- My Committee
 - Mary Hixon, PhD
 - James Padbury, MD
 - Carmen Marsit, PhD
- The Hixon Lab
 - Jeena Santos-Ahmed
 - Ben Moyer
 - Katie Dipalma
 - Linnea Anderson
 - Caitlin Brown
 - Jessica LaRocca
- Lab Alumni
 - Gregory Ouellet
 - Teresa Rasoulpour
- Center for Environmental Studies
 - Phil Brown, PhD
 - Steve Hamburg, PhD
- Marybeth Taub, RNC, BSN
 - Michelle Gottlieb
- Family and Friends
 - Jack and Patty Rogers
 - Chris Worley
 - CES Grad Students
 - Everyone on the fifth floor



Downstream Targets of NF- κ B/Akt

□ p21

- cyclin-dependent kinase inhibitor
- controls cell cycle progression
- linked to stress responses
- Previous data indicates increasing activity of p21-activated kinase 1 enhances stress induced NF- κ B activity (Friedland et al. 2007)



Akt1-deficient mice exhibit reduced mRNA and protein expression of p21

