



# Interspecific Effects of Amino-Dinitrotoluene Exposure



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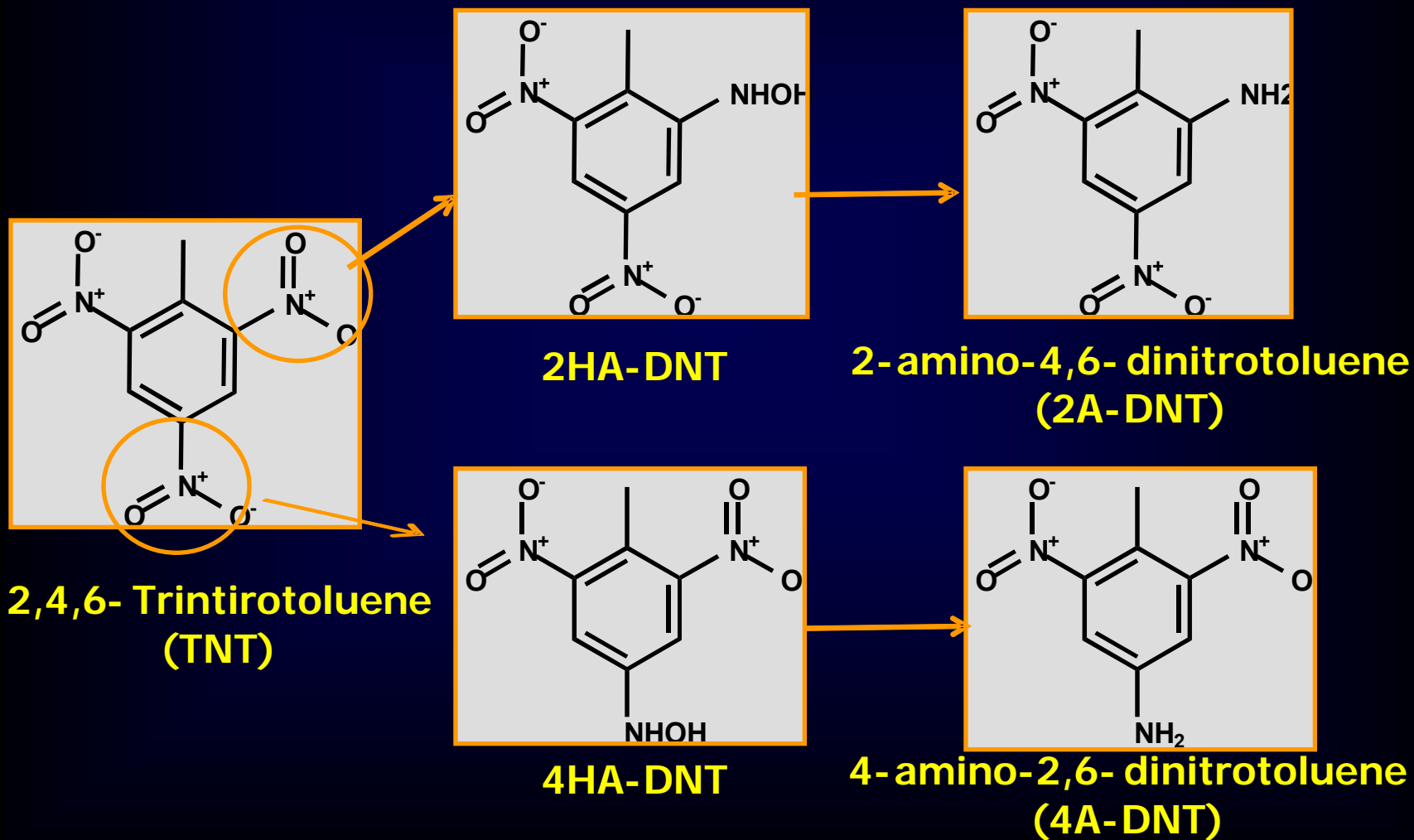


# Amino-Dinitrotoluene (A-DNT)

- Rapidly formed from TNT
- Primary anaerobic reduction metabolite of TNT
  - Bacteria facilitated
- In vivo bioaccumulation of A-DNT from TNT exposures
  - Earthworms
  - Salamanders
- Low water solubility and volatility
- Moderate mobility in soil
- Plants - highest concentration in roots

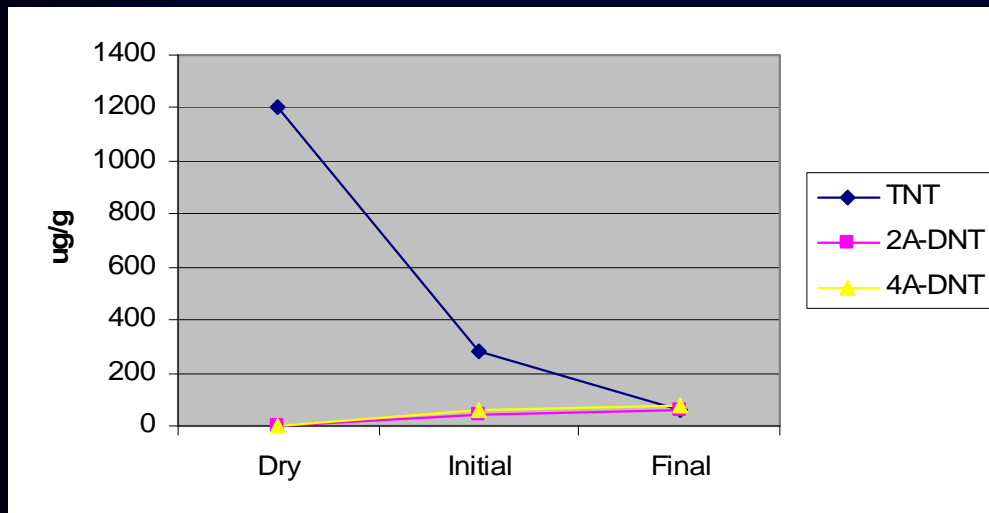


# Transformation Pathway

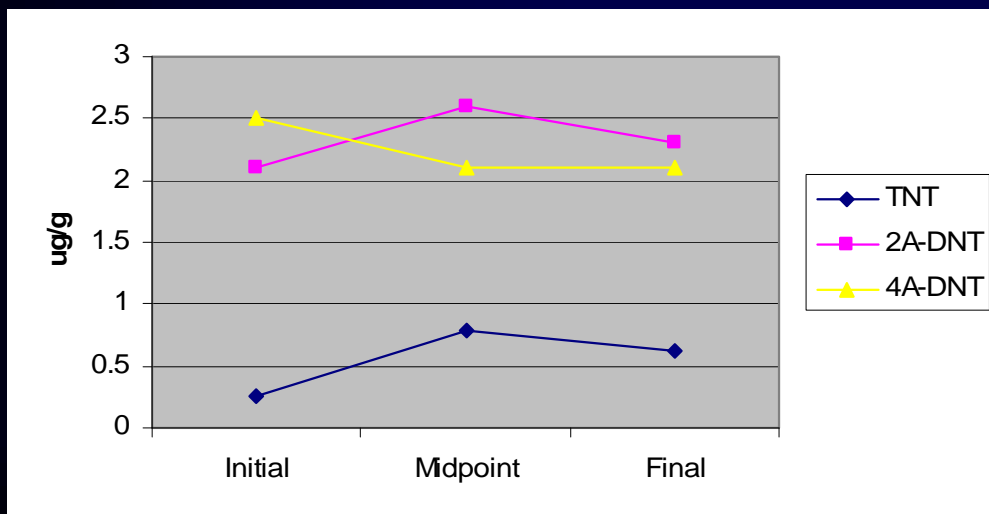


# Concentrations of TNT and primary breakdown products\*

## Soil



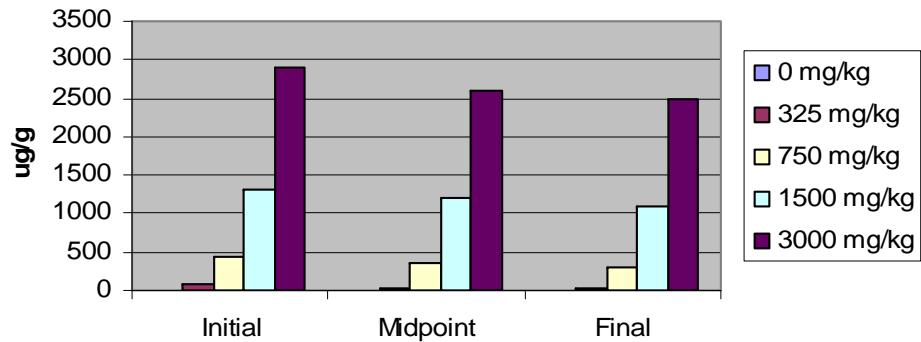
## Worms



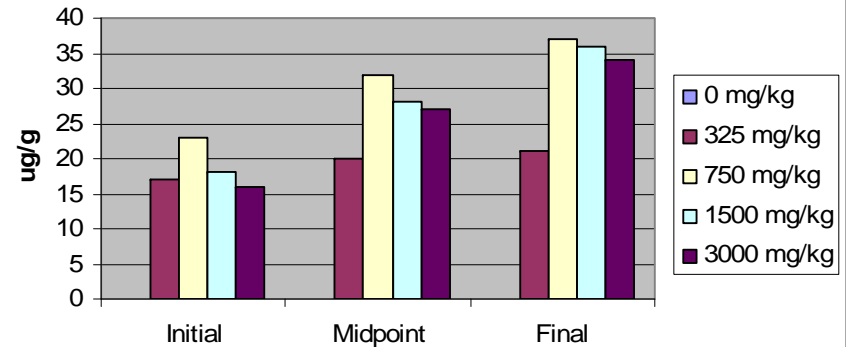
\*Johnson et al. 2000

# Soil concentrations of TNT (parent) and reduction products (Bazar et al. 2008)

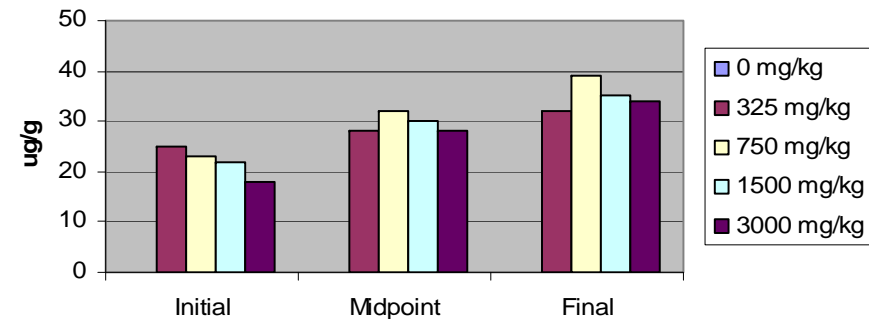
## TNT concentrations



## 2A-DNT concentrations



## 4A-DNT concentrations



# TNT transformation

- A nitro group on the TNT ring is rapidly reduced to an amine (2A-DNT or 4A-DNT)
- In vivo and in situ
  - In situ it is concentration dependant
  - In vivo it occurs at exposure point
    - TNT parent rarely found in tissue
    - Rate limiting secondary metabolic step
- 2A-DNT or 4A-DNT most prevalent in tissue of animals exposed to TNT
- Little plant uptake of parent or metabolites

# 2A-DNT Effects

**Mammals - Acute Only in Rats and Mice (LD50 rats 959-2240 mg/kg; mice 1342-1722 mg/kg).**

**Central nervous system excitability and/or depression, exaggerated reflexes, ataxia, delayed deaths to 10d after dosing, yellow-orange urine; 50% absorbed (Ellis *et al.* 1980).**

**Amphibians – Salamanders Soil [2A-DNT] and [4A-DNT] monitored with soil / earthworm TNT exposure - no adverse health effects observed (Johnson *et al.* 2000).**

**Earthworms - Toxicity 4A-DNT > TNT > 2A-DNT. 2A-DNT bioaccumulated most and needs consideration when evaluating overall TNT toxicity (Lachance *et al.* 2004).**

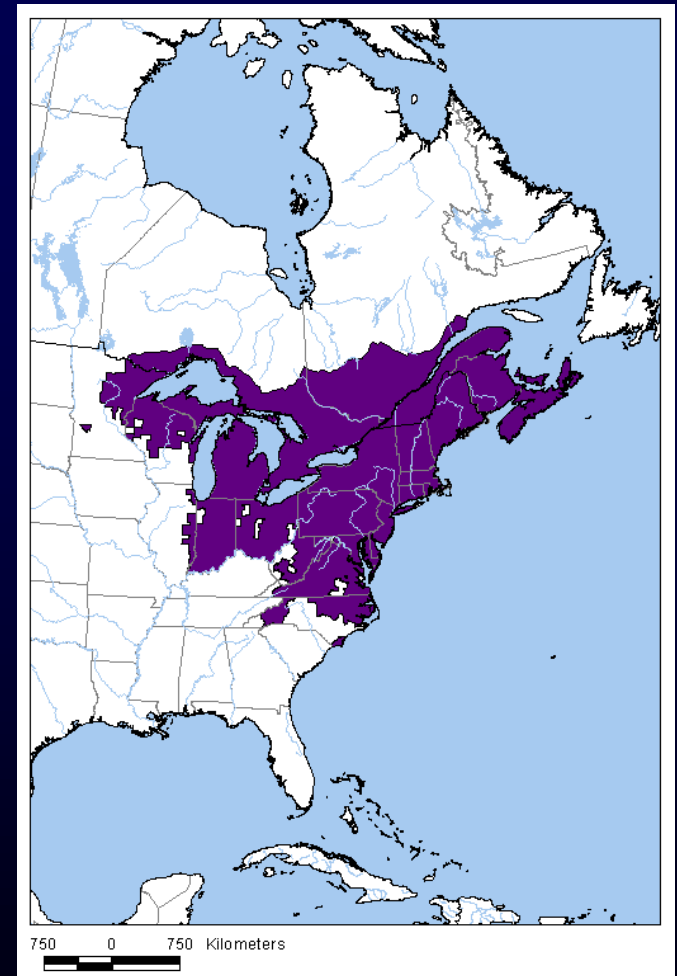
**Birds - Northern Bobwhite (*Colinus virginianus*)**

**Reptiles – Western fence lizard (*Sceloporus occidentalis*)**

**Amphibians – red-backed salamander (*Plethodon cinereus*)**

# Soil amphibian model

- *Plethodon cinereus*
- Lungless
- Thin integument
- Terrestrial
- Long-lived
- Small home range
  - (0.16-0.33m<sup>2</sup> (Petranka 1998))



# Salamander Toxicity Test

- 28-d exposure
- Blood parameters evaluated
- Histopathological examination
- Biomarkers

- Soils spiked at 4 concentrations + control
- Fed *Drosophila*
- Weighed weekly
- Fed every other day
- Observed daily



# A-DNT soil exposures to *P. cinereus*

- 10d range finding study
- 2A-DNT and 4A-DNT
  - 2A-DNT more toxic
    - No mortality to 10k mg/kg, but greater occurrence of overt symptoms.
- 28d subchronic 2A-DNT study
  - <0.05, 34, 173, 603, 1533 mg/kg (dry)
  - No mortality\*
  - Lethargy, unresponsiveness, inappetence, adverse behavior at 603-1533 mg/kg; adverse hematology at 1533 mg/kg.

\*One death at 603 mg/kg not thought to be compound related  
(Bazar et al. *in prep*).

# *Sceloporus* as a Reptile Model

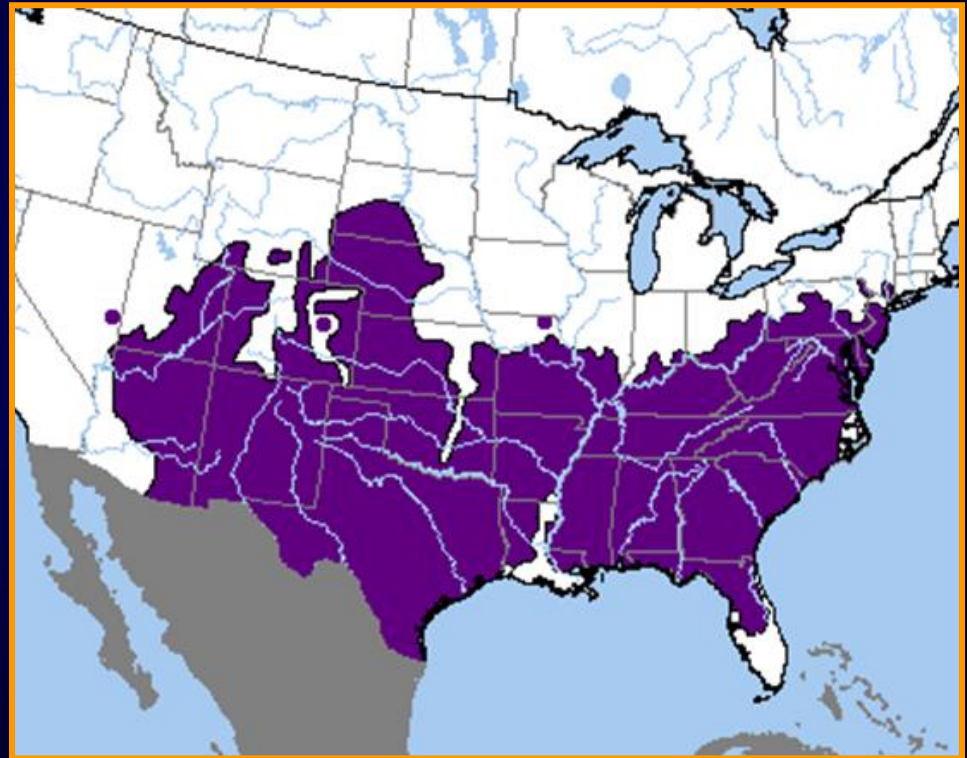
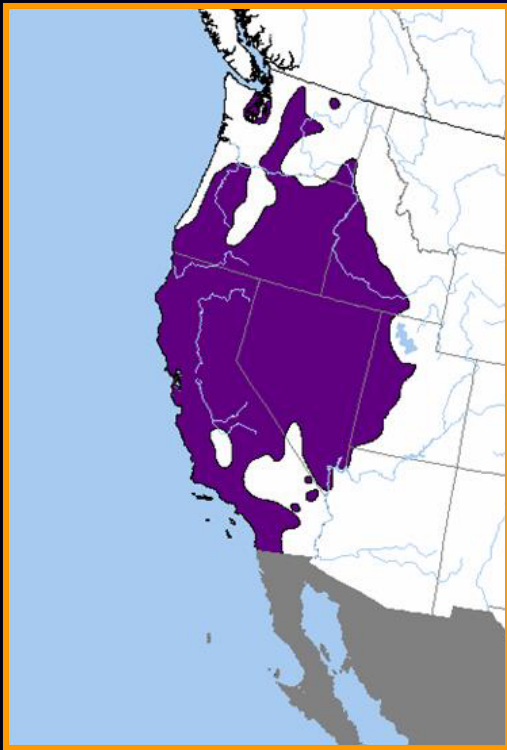
- > 70 species from northern U.S. to Panama
- Sea level to > 4000 m, deserts to subalpine forests
- Terrestrial habitats vary geographically
- Size varies from 4 to 60gm (15 - 25g, 20 – 25cm total length)
- Diurnal, hibernates / aestivates
- Invertivore – insects - beetles, flies, ants, spiders, snails



# U.S. Range Maps

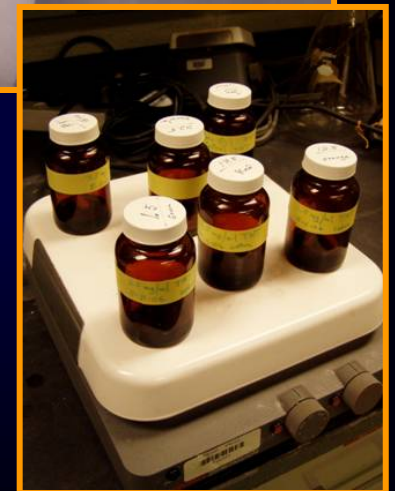
## Western & Eastern Fence Lizards

(*Sceloporus occidentalis* / *undulatus*)

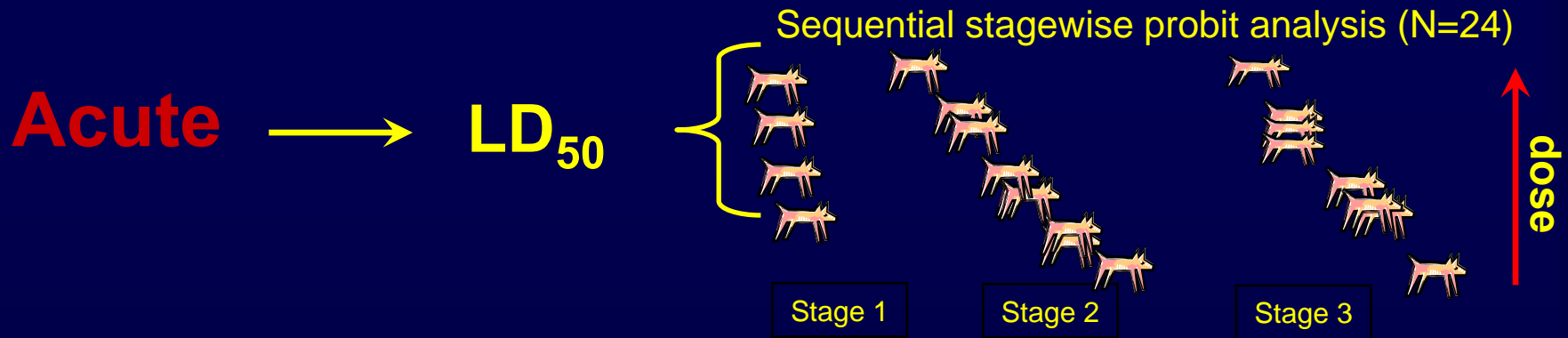


# Basic Study Design

- Acute lethal ( $LD_{50}$ )
- Subacute (14-Day)
- Subchronic (60-Day)



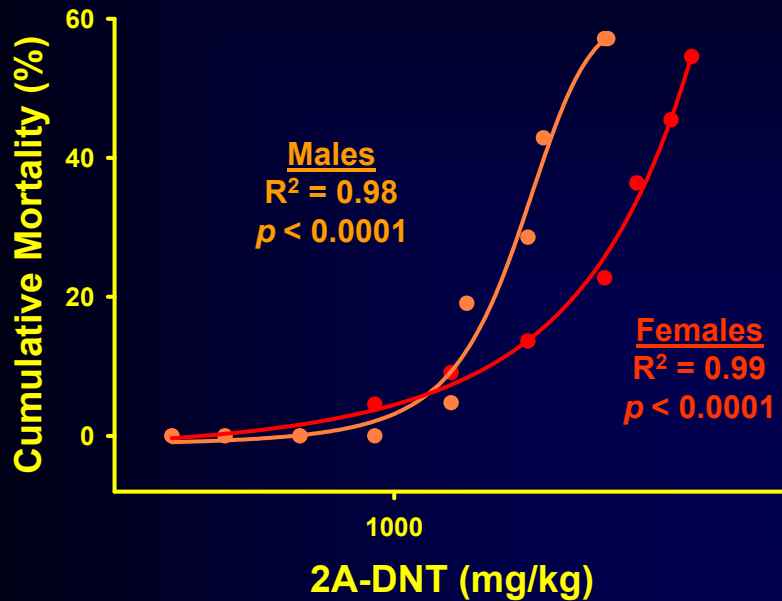
# Technical Approach



5-treatments  
(4 doses+1control)  
12/sex/treatment



# Acute Toxicity



Cumulative Animal Mortality  
LD<sub>50</sub> - no sex difference

Males = 1406 (947, 2087)

Females = 1867 (1076, 3237)

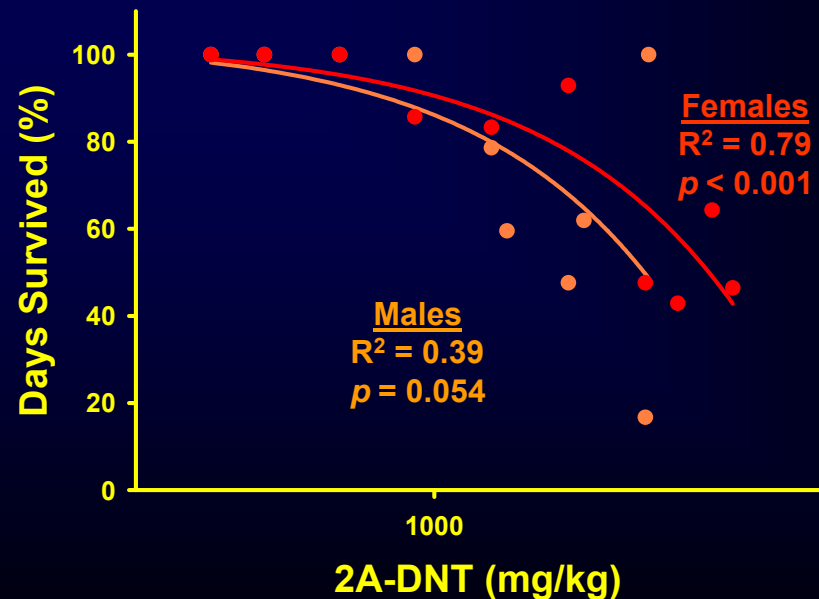
Depression, weakness, anorexia,  
weight loss, yellow vent and feces

Percent days survived decreased  
with increasing dosage - no sex  
difference.

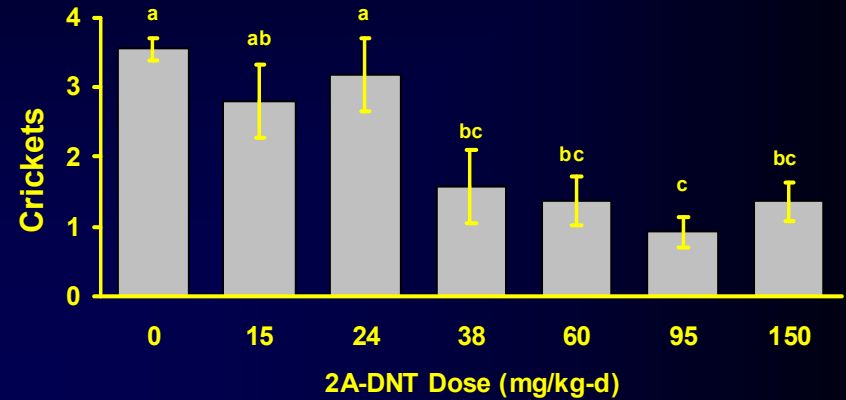
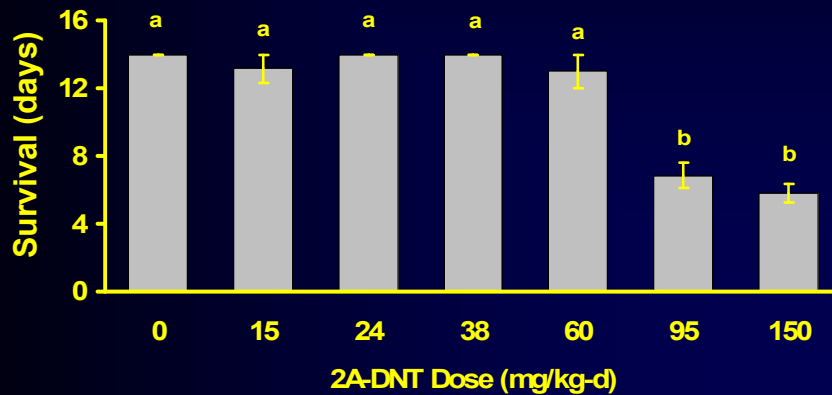
Average (days):

Males =  $9.29 \pm 1.10$

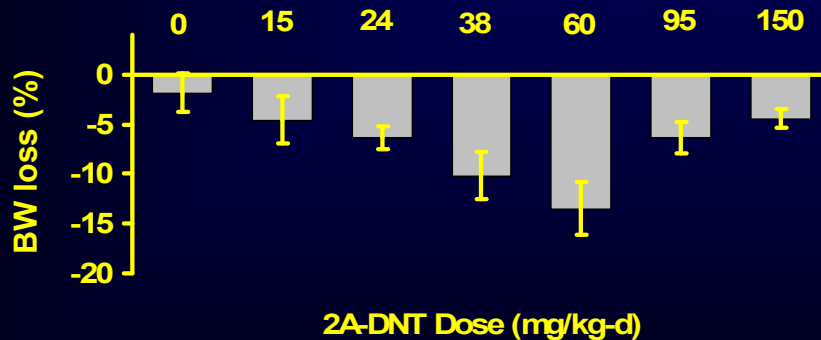
Females =  $9.91 \pm 0.99$



# Subacute (14-Day)

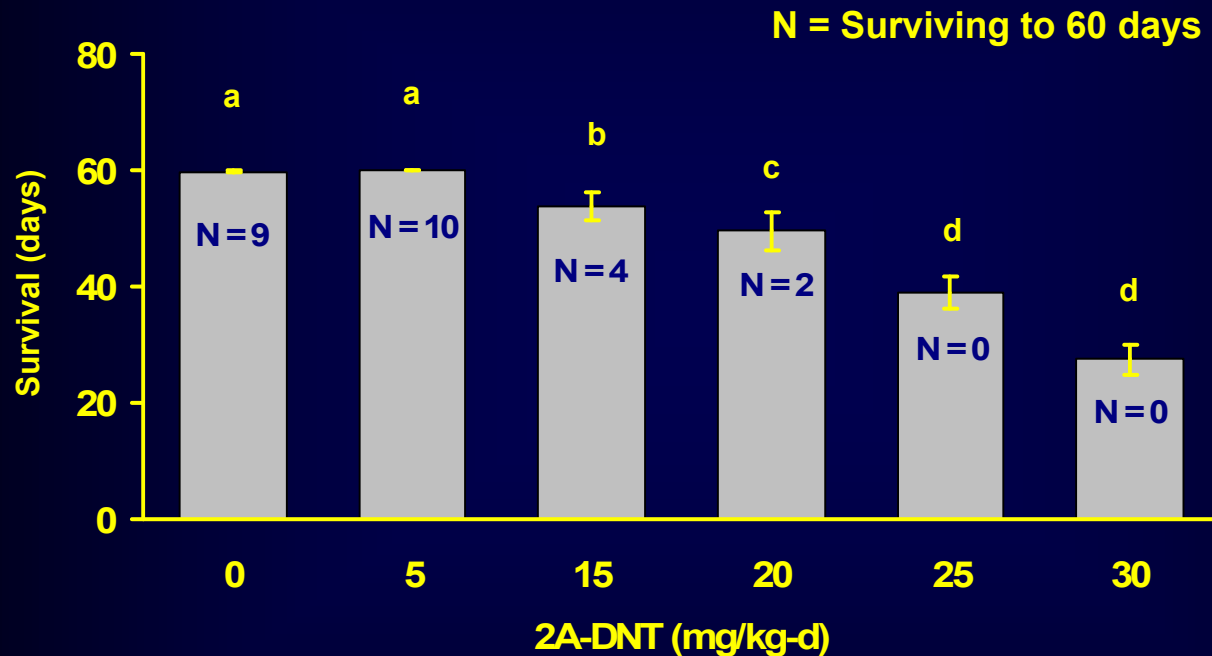


Significant effects on survival at  $\geq 95$  mg/kg-d. Two highest groups survived approx. 7 and 6 days.



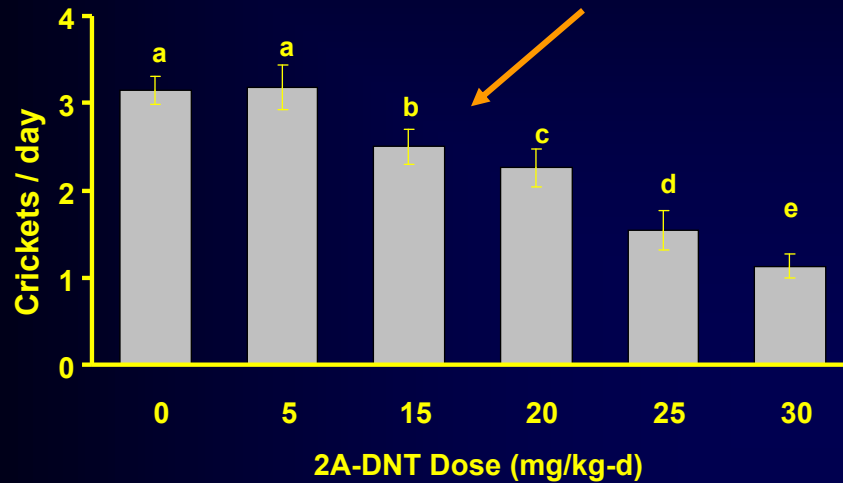
Loss of BW was dose-related in the lower exposures due to approximate 14-d survival times.

# Subchronic (60-Day)



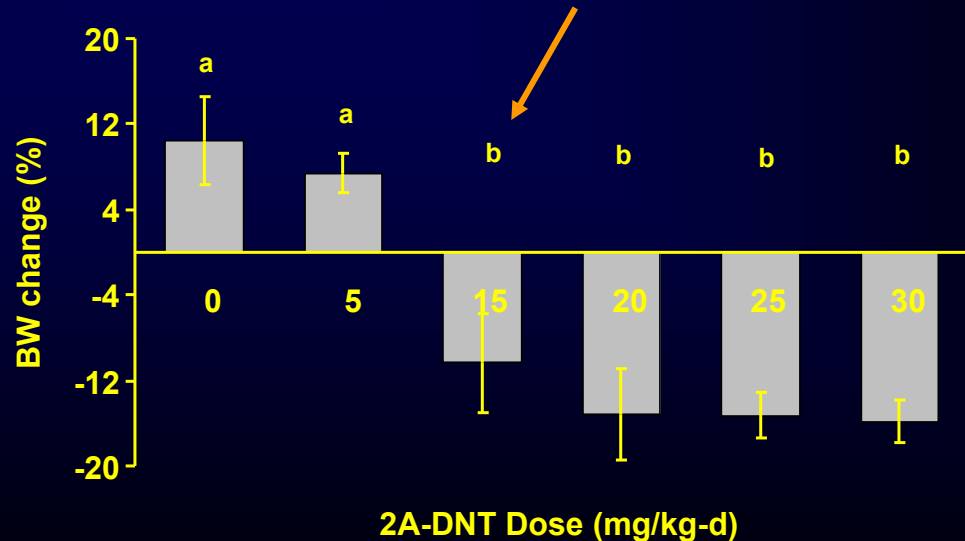
Survival time and number of lizards per group decreased significantly at  $\geq 15$  mg/kg-d in the 60-day experiment.

# Subchronic (60-Day)

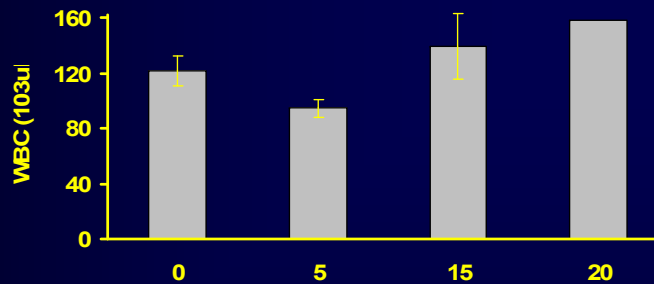
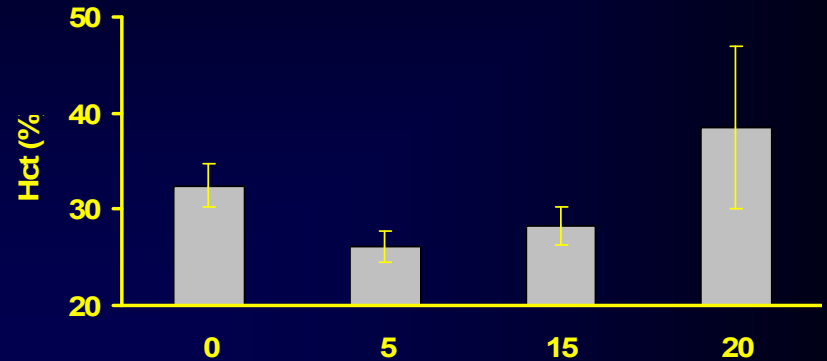
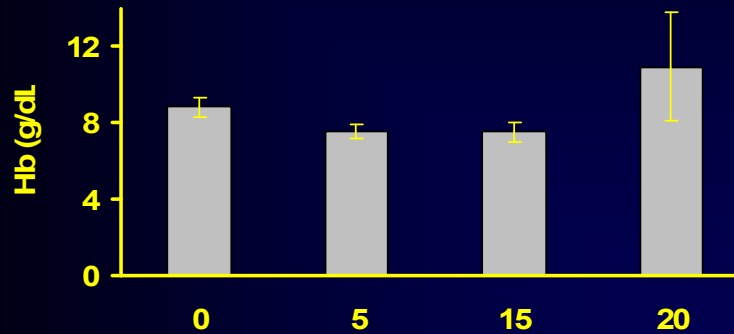


Reduced feeding rates were statistically significant at  $\geq 15$  mg/kg-d.

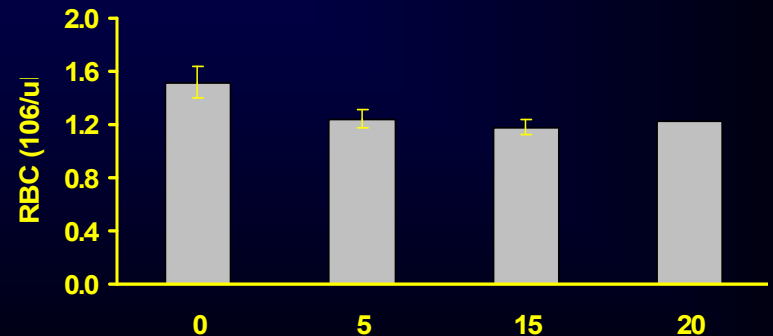
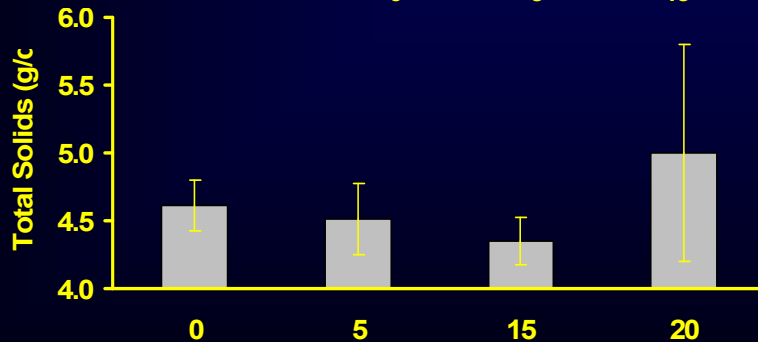
Net loss in BW at 15 mg/kg-day, coincident with a daily cricket intake of 2.5. Change in BW compared to Day 0.



# Subchronic (60-Day)



No significant differences in measured hematologic endpoints.



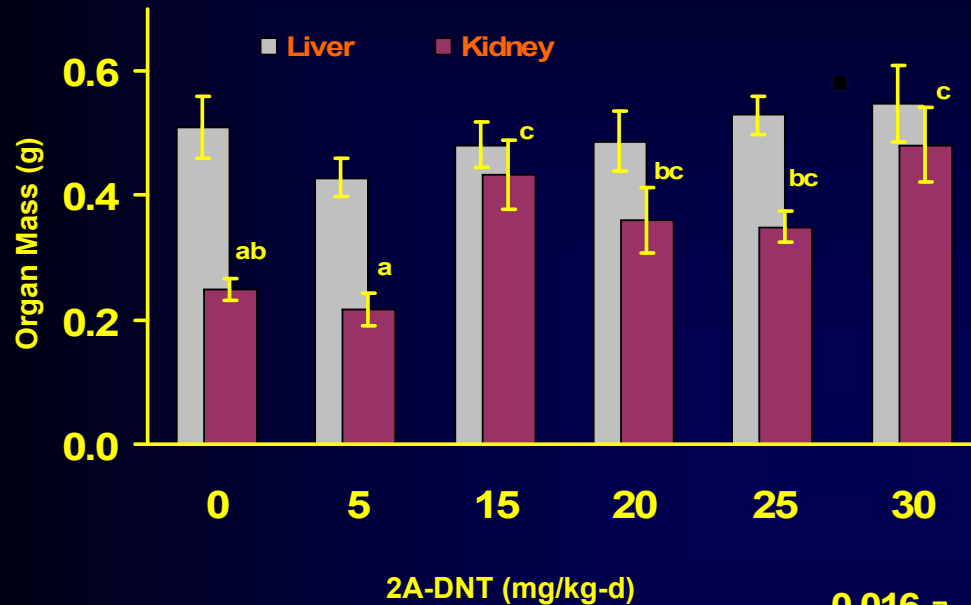
# Subchronic (60-Day)

## 2A-DNT Dose (mg/kg-d)

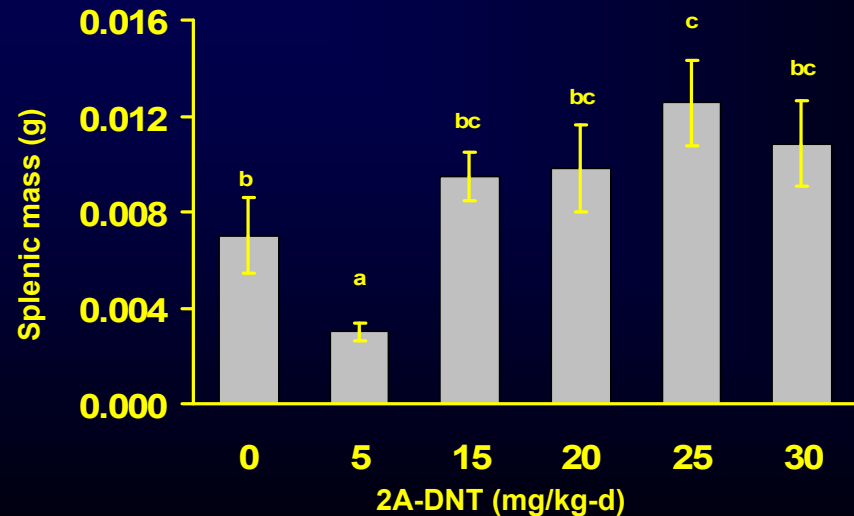
	0	5	15	20
ALB (g/dL)	2.01 +/- 0.20	1.64 +/- 0.23	1.40 +/- 0.18	1.60
ALKP (U/L)	34.00 +/- 4.04	25.57 +/- 5.38	34.67 +/- 10.18	45.00
AST (U/L)	104.17 +/- 11.42	64.14 +/- 11.67	80.67 +/- 19.72	121.00
BUN (mg/dL)	2.67 +/- 0.21	2.86 +/- 0.14	3.75 +/- 0.48	6.00
Creatinine (mg/dL)	0.53 +/- 0.08	0.64 +/- 0.09	0.73 +/- 0.29	1.10
Phosphorus (mg/dL)	10.26 +/- 0.64	8.45 +/- 0.47	13.83 +/- 2.88	11.80
Uric Acid (mg/dL)	1.31 +/- 0.26	1.33 +/- 0.25	1.90 +/- 0.99	2.00

Values : Mean ± SEM

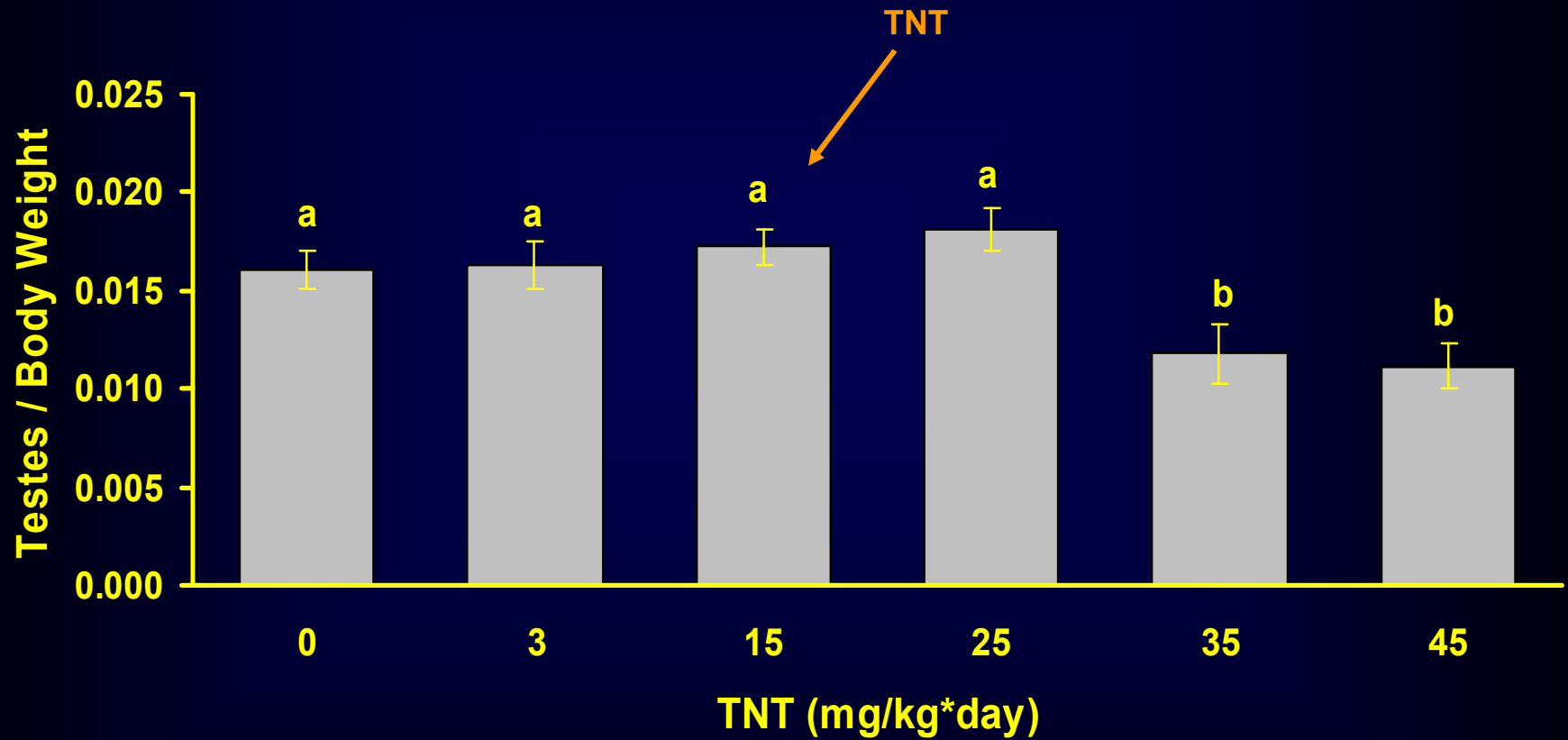
# Subchronic (60-Day)



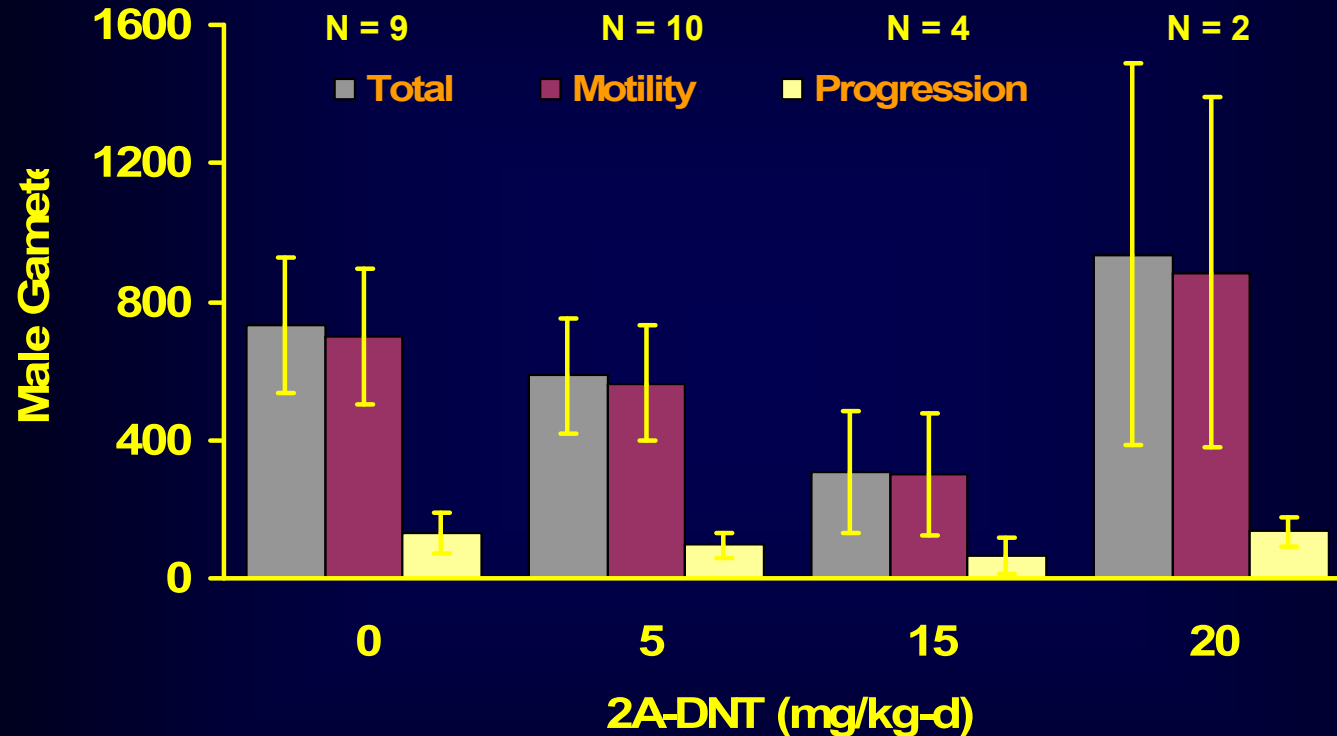
No difference in liver mass.  
Kidney enlarged at  $\geq 15$  mg/kg-d.



# Testes mass



# Sperm Production



Non-significant differences in total numbers, motility, or progression of male gametes.

# Summary

- *S. occidentalis* is a useful laboratory model for toxicological investigation in reptiles.
- Clinical signs were non-specific and best characterized by anorexia, cachexia and yellow-orange discolored vent.
- Kidney effects; liver effects at levels where mortality occurred.
- LOAEL / NOAEL were determined at 15 / 5 mg/kg-d  
(Based on survival, body weight, food intake, kidney effects)

# Northern Bobwhite



- Oral acute (stagewise probit)
- Oral subacute (14d range finding)
  - 0, 50, 125, 265, 550, 1000 mg/kg-d
- Oral subchronic (60d gavage)
  - 0, 0.5, 3, 14, 30 mg/kd-d

# Acute Study - results

LD50 = 1167 mg/kg

Fieller's 95%CI (356,1466)

Delta 95% (942, 1445)



# Sub-acute Study - methods

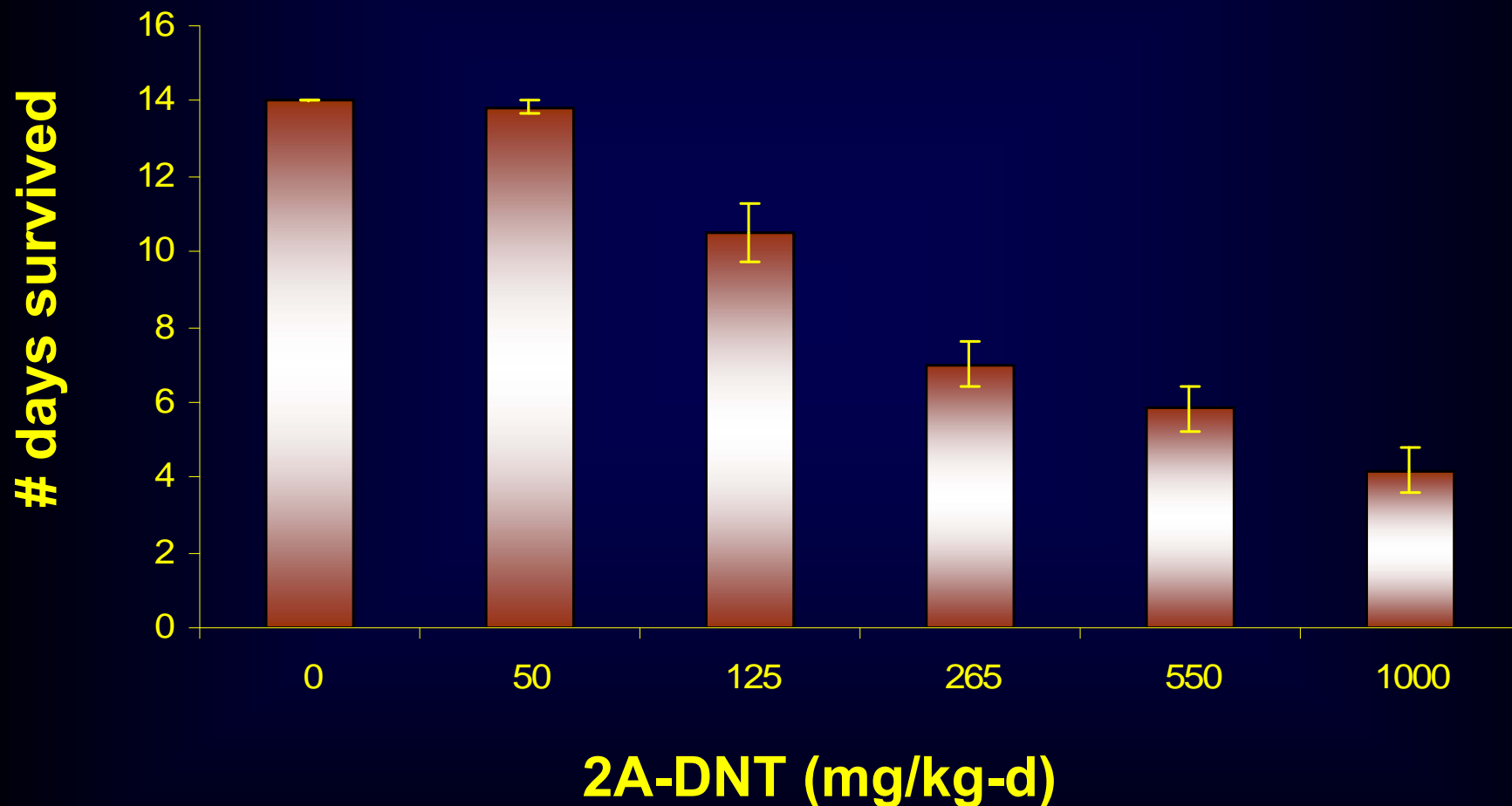
Daily gavage, 14 d

Treatment levels – 0, 50, 125, 265, 550, 1000 mg/kd-d

Measurements:

- mortality
- body weights
- organ weights
- gross observations

# Sub-acute Study - results



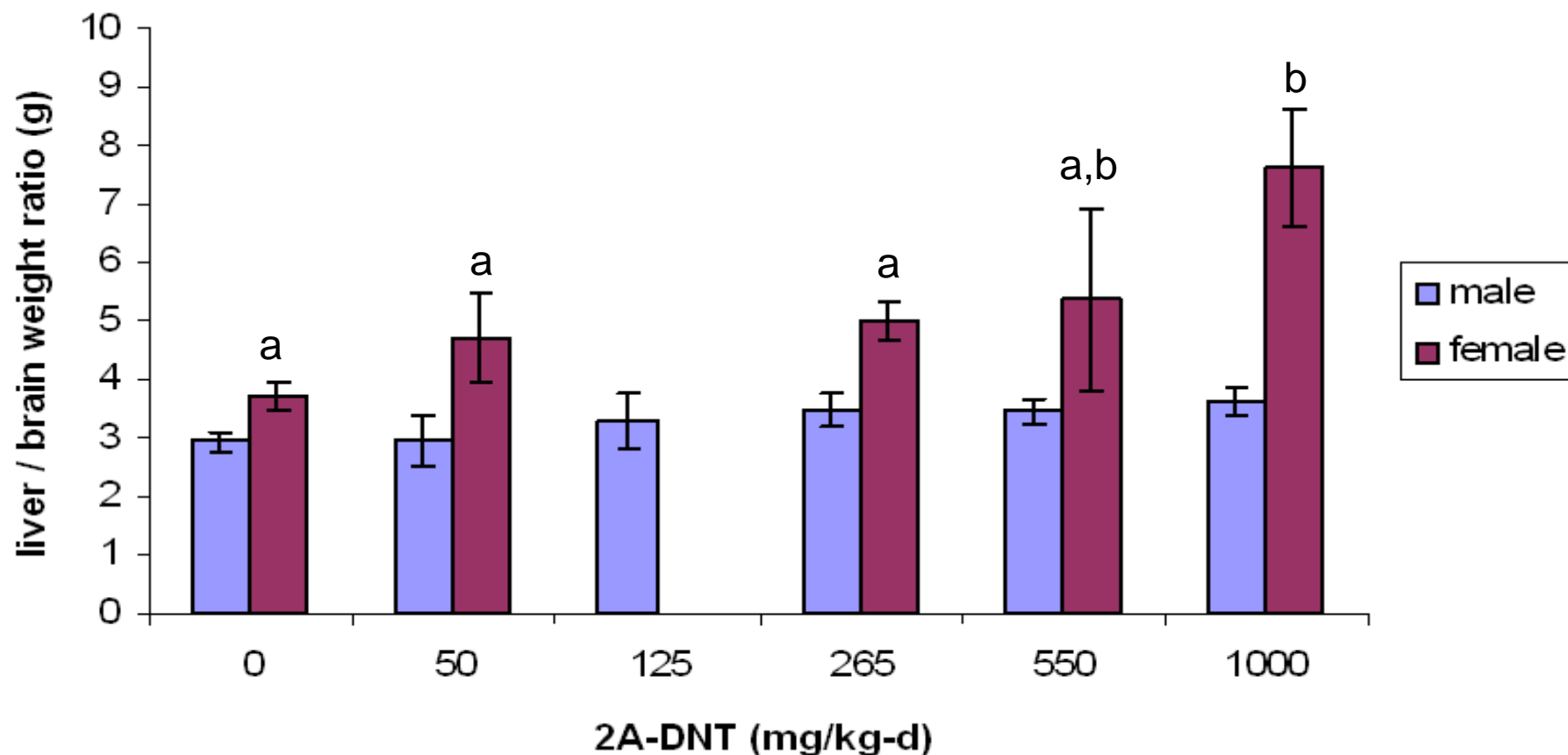
# Sub-acute Study - results

- No significant differences in body weights among treatments.
- Significant differences in liver and spleen : brain weight ratios among treatments for females only.

organ	treatment
brain	no
heart	no
liver	yes (f)
kidneys	no
spleen	yes (f)
gonads	no

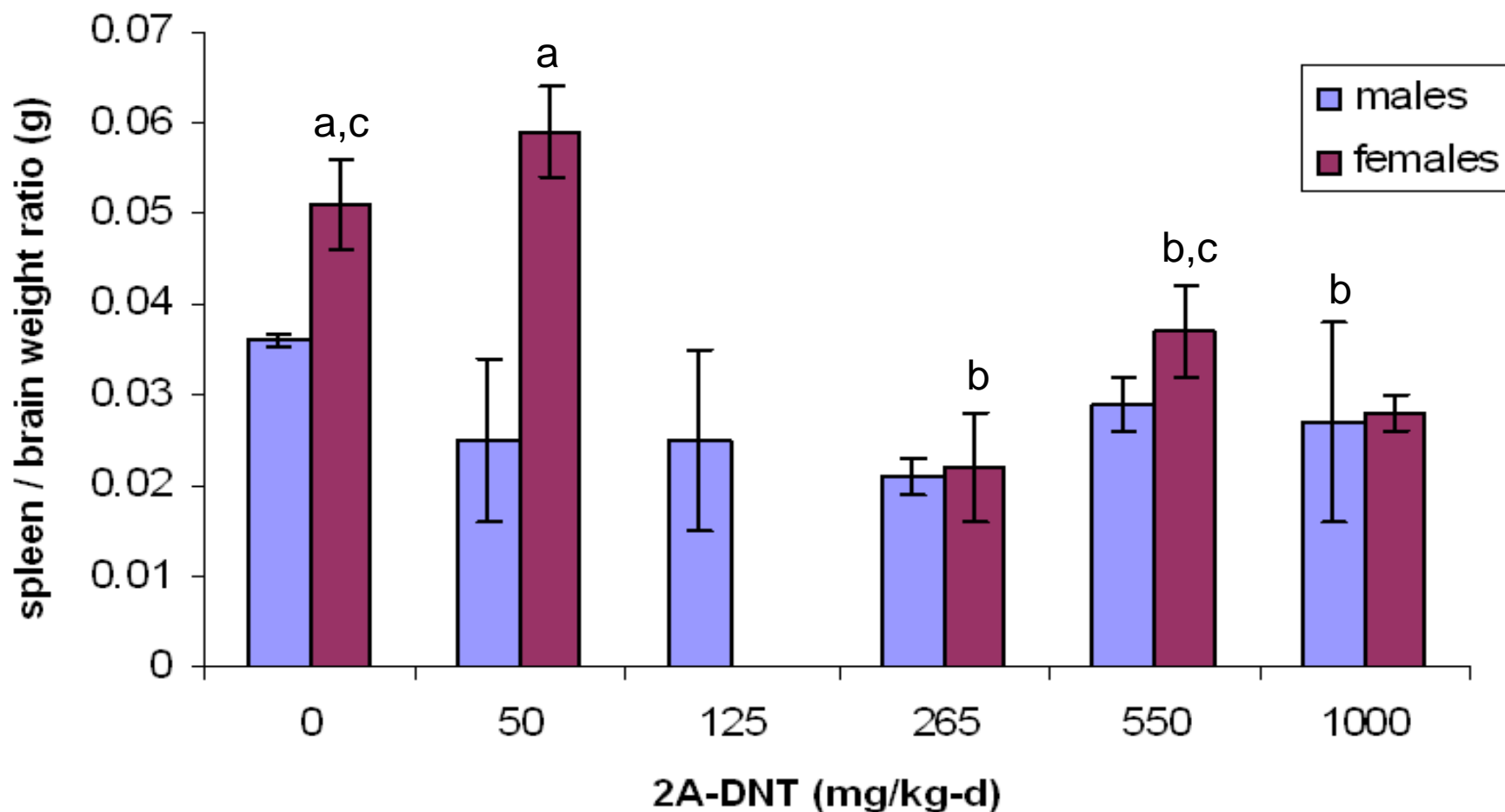
# Sub-acute Study - results

## Liver : Brain Weight Ratios



# Sub-acute Study - results

## Spleen : Brain Weight Ratios



# Sub-acute Study - results

Consistent signs upon necropsy:

- enlarged gall bladder
- green food contents in gizzard
- no food in lower GI, although crop full
- scant white feces (urates only)



# Sub-chronic Study - results

Significant differences in feed consumption in both genders.

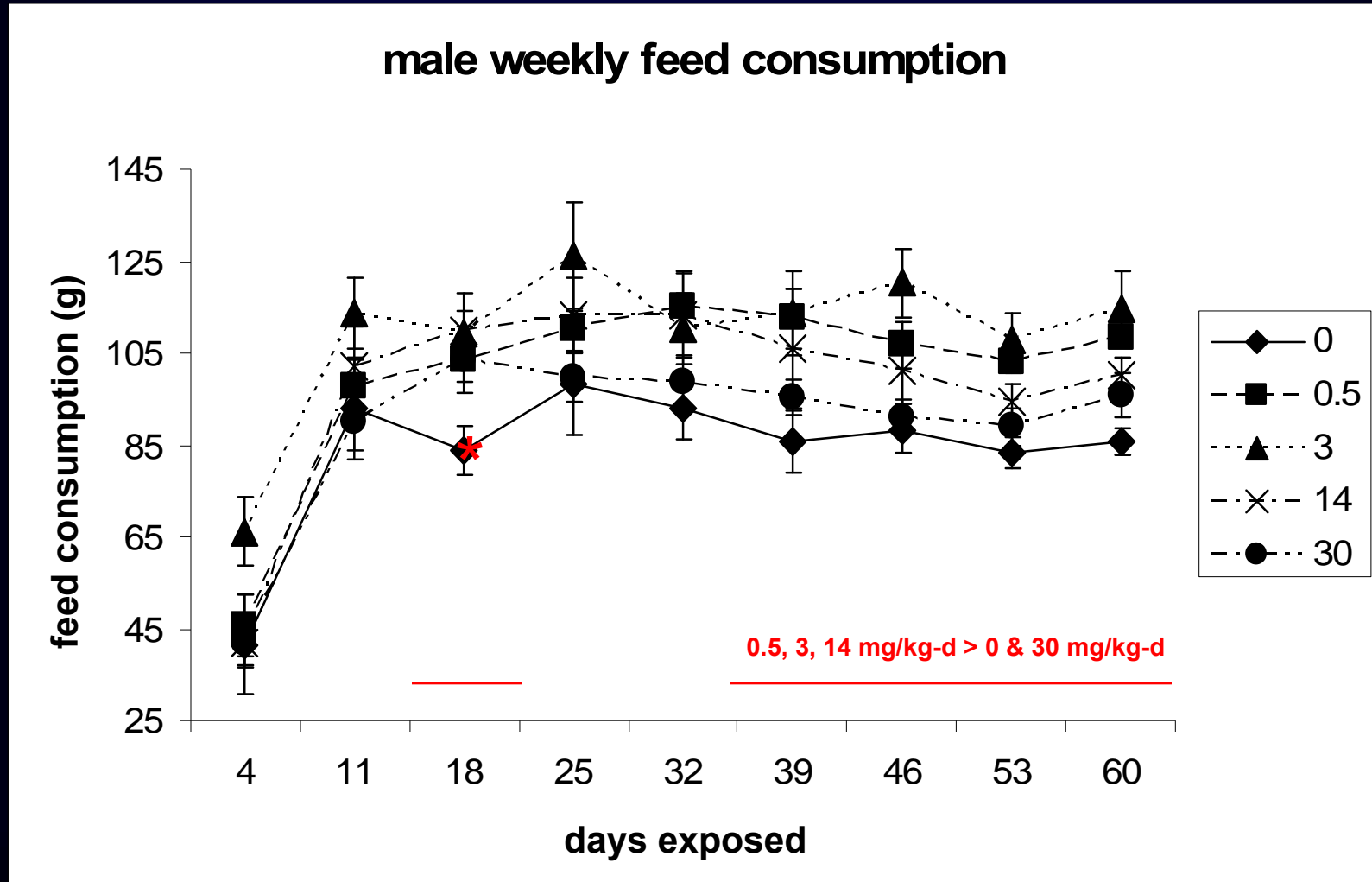
No significant differences in body weights among treatments.

Significant differences in liver : brain weight ratios among treatments for both genders.

organ	treatment
brain	no
heart	no
liver	yes
kidneys	no
spleen	no
gonads	no

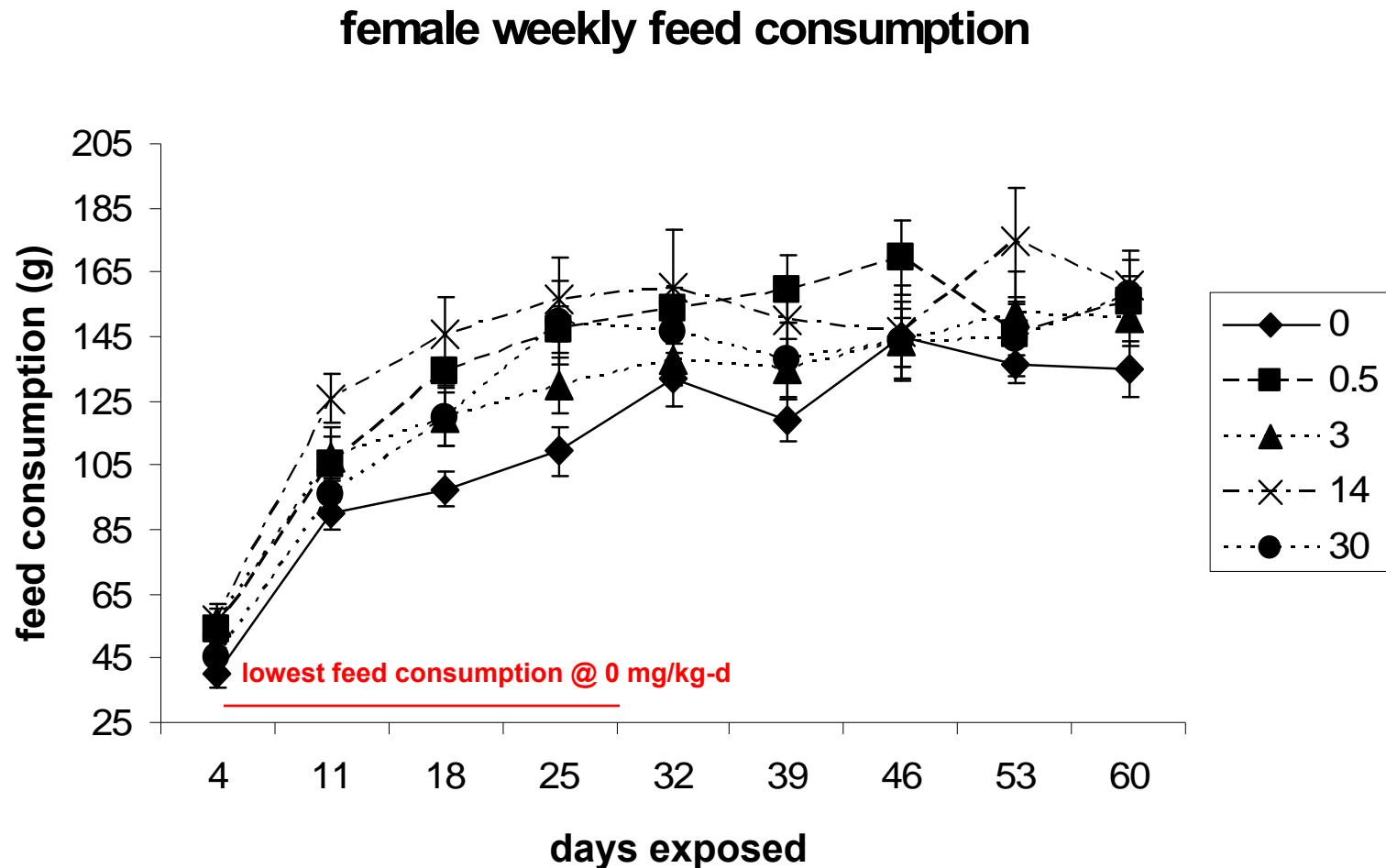
# Sub-chronic Study - results

## Weekly Feed Consumption



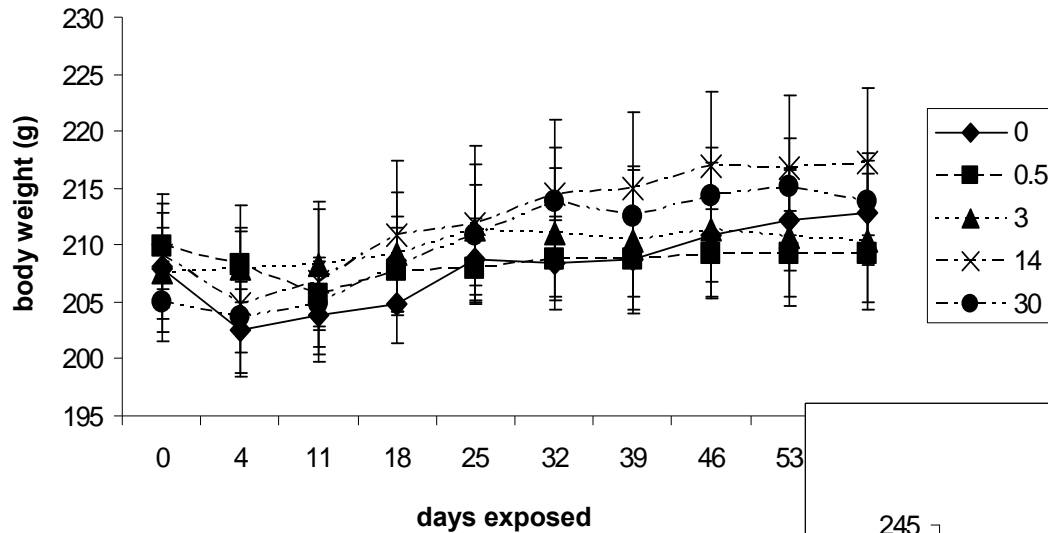
# Sub-chronic Study - results

## Weekly Feed Consumption



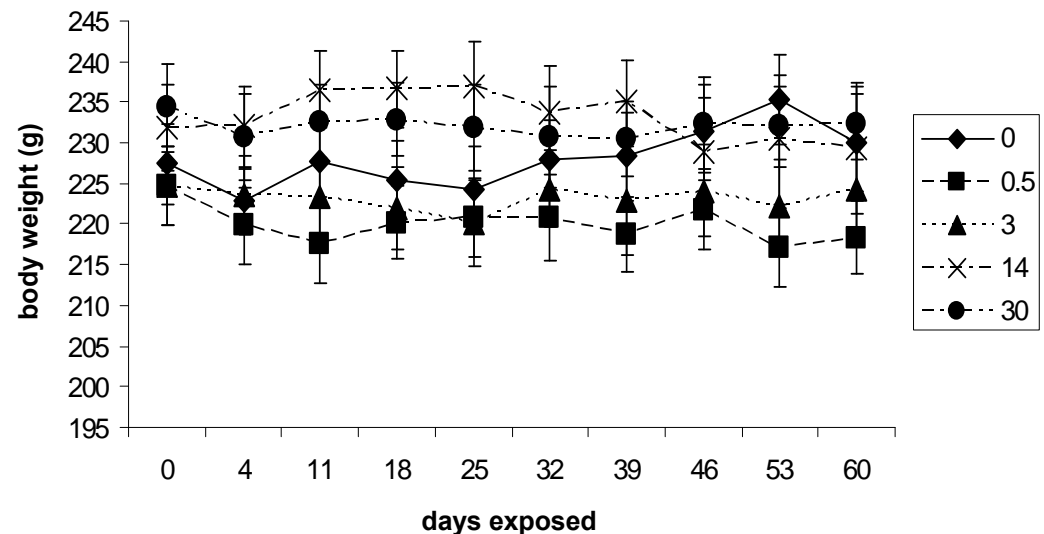
# Sub-chronic Study - results

male weekly body weights



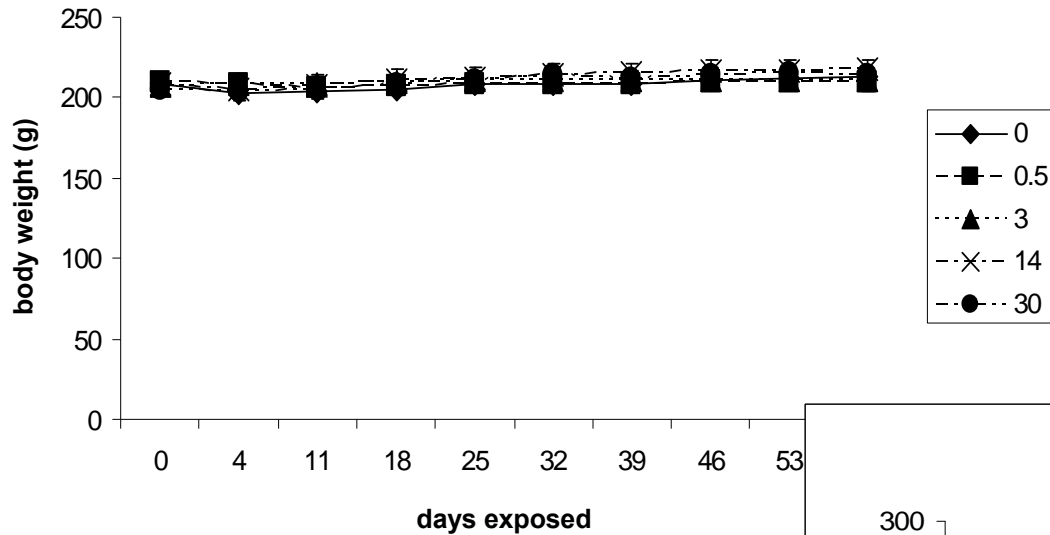
## Weekly Body Weights

female weekly body weights



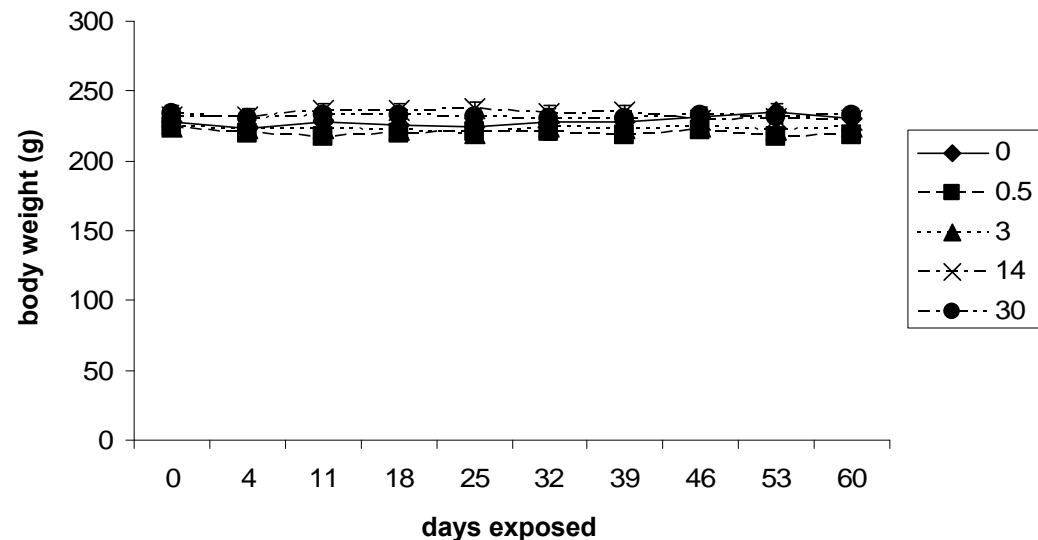
# Sub-chronic Study - results

male weekly body weights



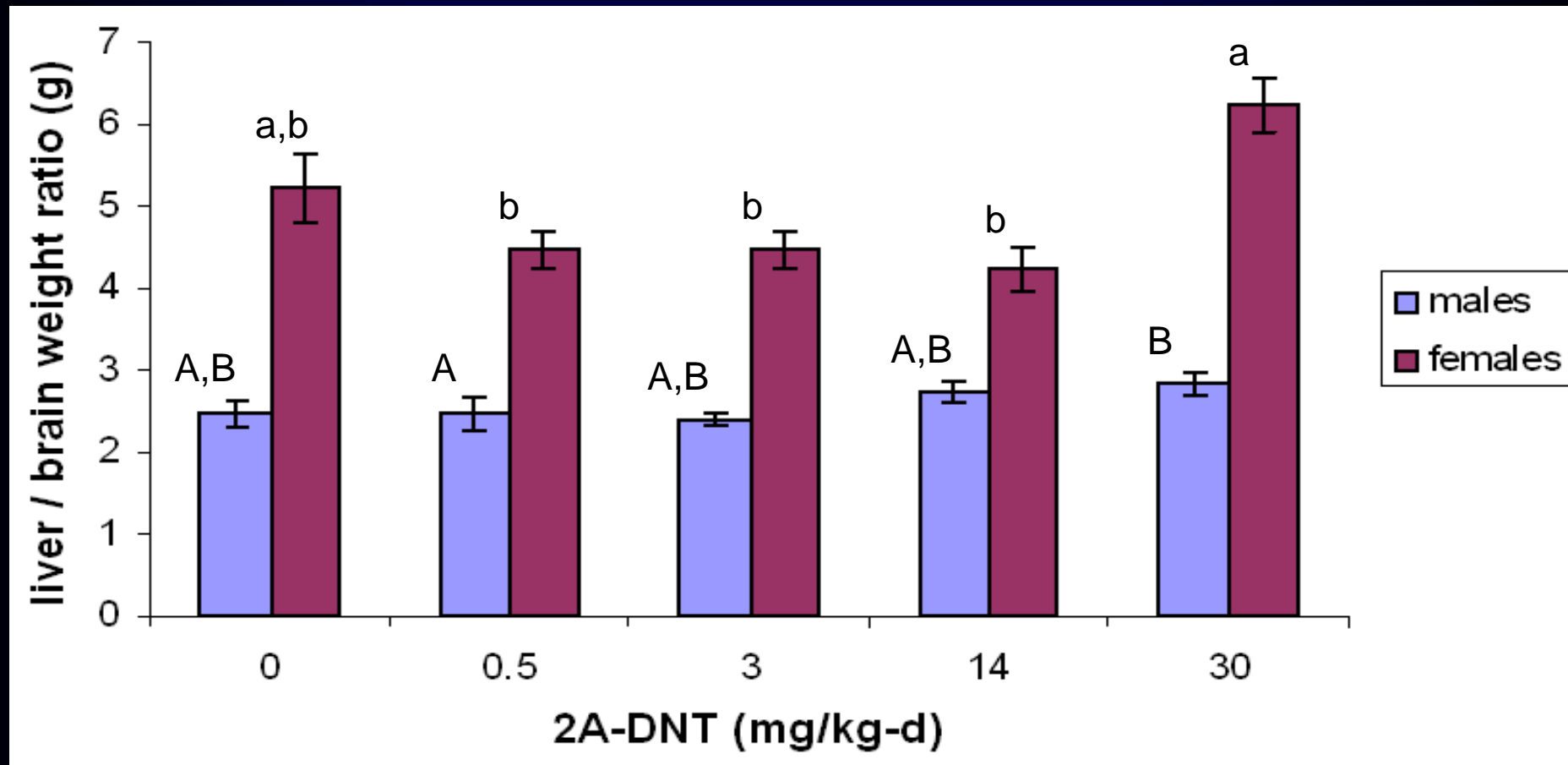
## Weekly Body Weights

female weekly body weights



# Sub-chronic Study - results

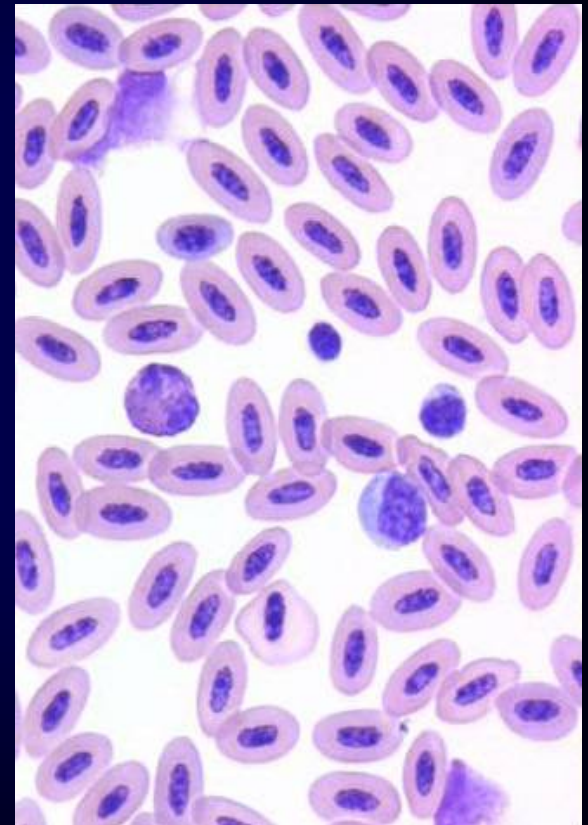
## Liver / Brain Weight Indices



# Sub-chronic Study - results

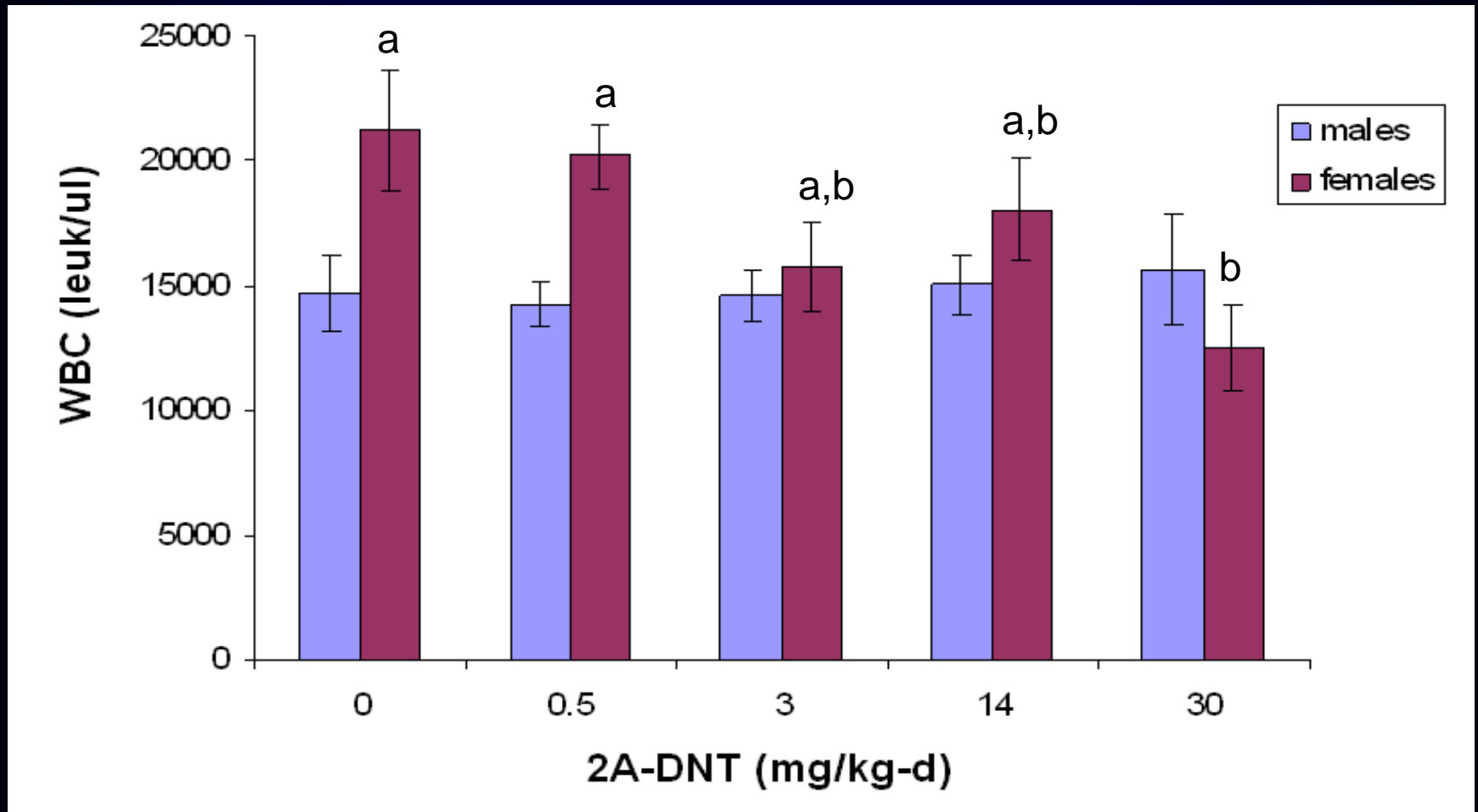
Hematology results:

	Effect?
Hemoglobin	no
Hematocrit	no
Total solids	no
Erythrocytes	no
Leukocytes	yes - females only



# Sub-chronic Study - results

## Total leukocytes (WBC)



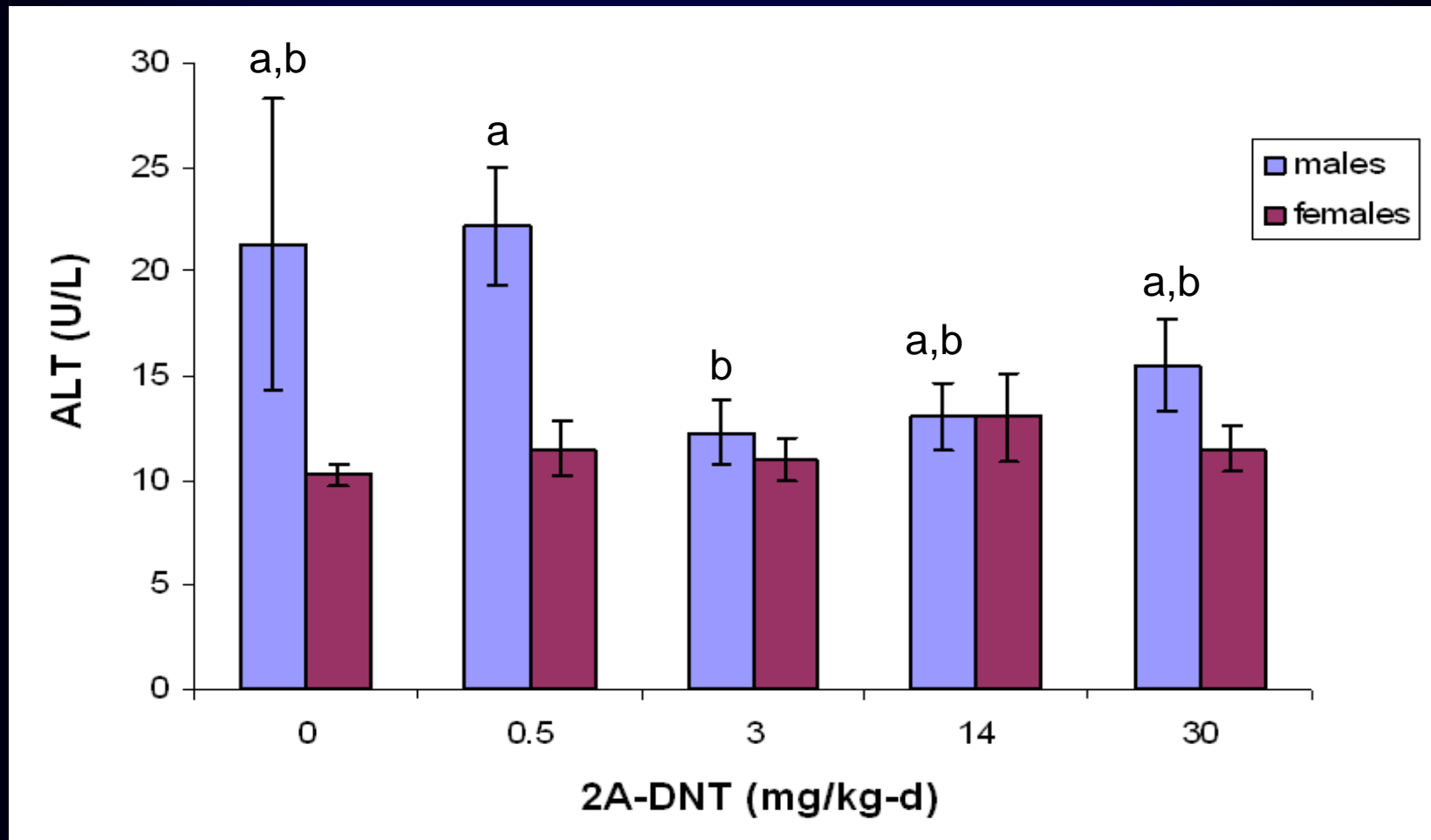
# Sub-chronic Study - results

## Blood chemistry results:

	Effect?
Alkaline phosphatase	no
Alanine aminotransferase	yes - males only
Aspartate aminotransferase	no
Calcium ion	no
Creatine kinase	no
Total protein	no
Globulin	no
Albumin	no
Lactate dehydrogenase	no
Phosphate	no
Triglycerides	yes, males only
Uric acid	no
Sodium ion	no
Potassium ion	no
Chlorine ion	no

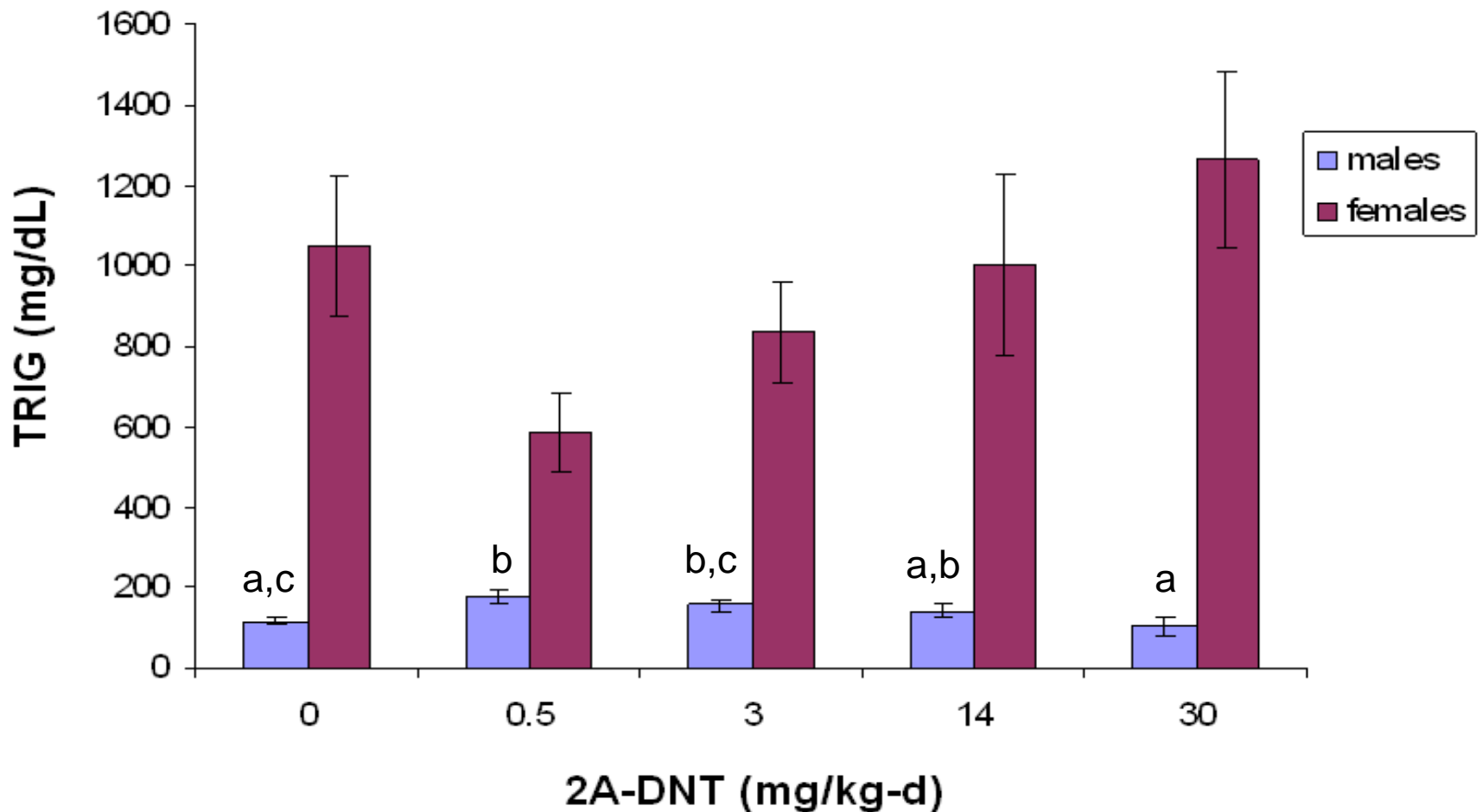
# Sub-chronic Study - results

## Alanine aminotransferase (ALT)



# Sub-chronic Study - results

## Triglycerides (TRIG)



# Summary subchronic LOAELs/NOAELs

	TNT	A-DNT	Targets (TNT/ADNT)
Amphibians	373 472	603 173	Blood Behavior
Reptiles	25 15	15 5	Blood Kidney
Birds	70 20	30 14	Kidney/liver Liver

# Summary

- ADNT is primary TNT metabolite
- Effects from ADNT exposure are different from TNT
  - Differences between species
  - ADNTs are less toxic from acute exposures than TNT, however,
  - ADNTs are more toxic than TNT from subchronic exposures
    - Different sequelae, different endpoints

# Acknowledgements

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SERDP ER-1420***