



**NTP**  
National Toxicology Program

# Toxicology and Carcinogenesis Studies of Ethinyl Estradiol in Sprague-Dawley Rats

TR 548





## Introduction – Ethinyl Estradiol Chronic Study

- Steroidal estrogens, including ethinyl estradiol, are listed as known human carcinogens
  - Chronic safety studies with higher doses have been conducted previously
  - Aim of the present study is to evaluate chronic low exposure dose effects, reversibility of effects, and potential generational carryover effects
- Study utilizes NCTR Sprague-Dawley rats, 5K96 diet, and exposures from conception
  - Vast historical control database from NTP F344 rat studies not applicable
  - One study (genistein, TR 545) conducted under identical conditions with two control groups is available for comparison

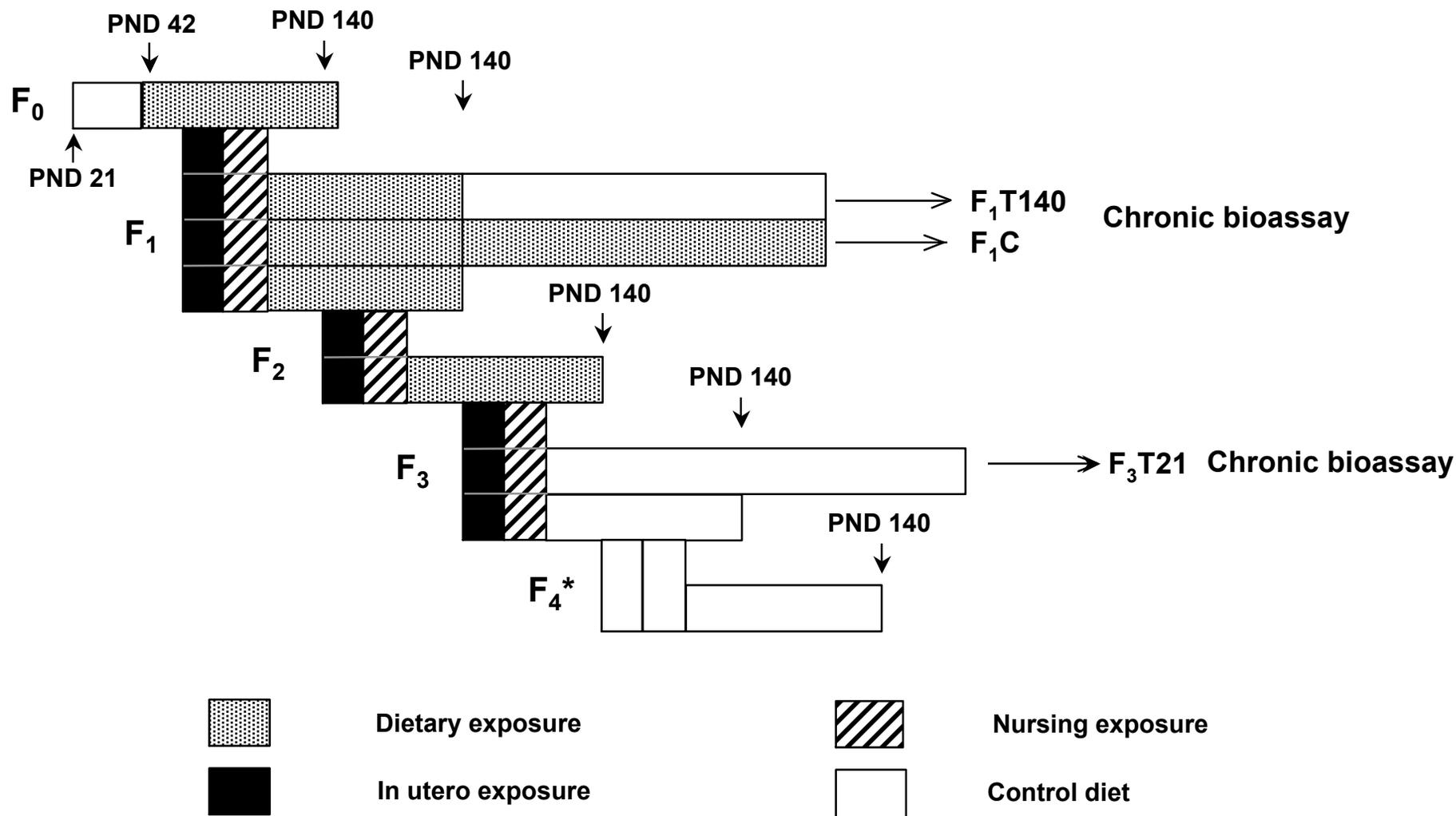


## Chronic Study Design

- Dosing (chronic phase)
  - $F_1$ 
    - Window 1: Continuous dosing from conception to 2 yrs. ( $F_1C$ )
    - Window 2: Dosing from conception to 20 weeks, then control diet to 2 yrs. ( $F_1T140$ )
  - $F_3$ 
    - Offspring of two prior generations of animals exposed to ethinyl estradiol
    - Dosing from conception to PND 21, then control diet to 2 yrs. ( $F_3T21$ )
- 50 animals per sex per dose group per study arm (one control group for the  $F_1$  study arms)



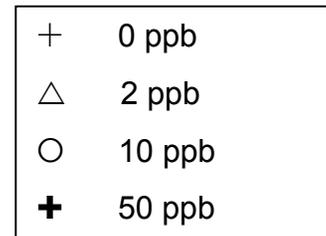
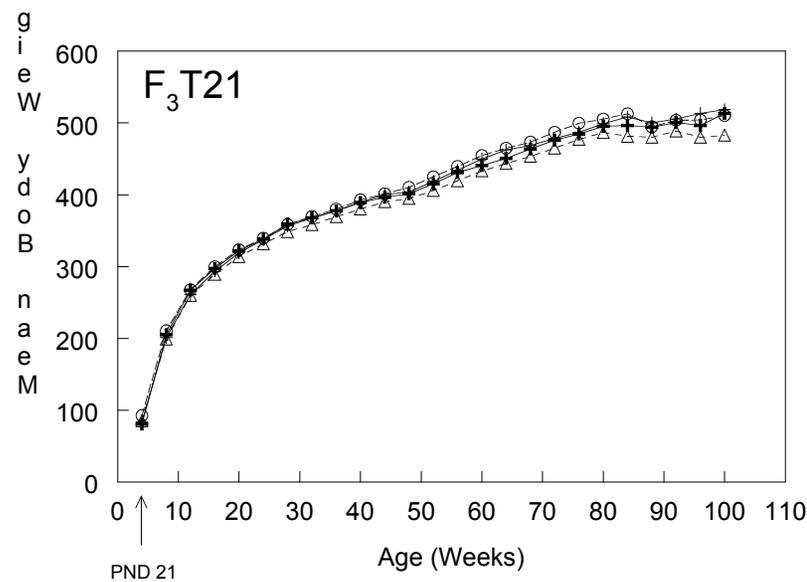
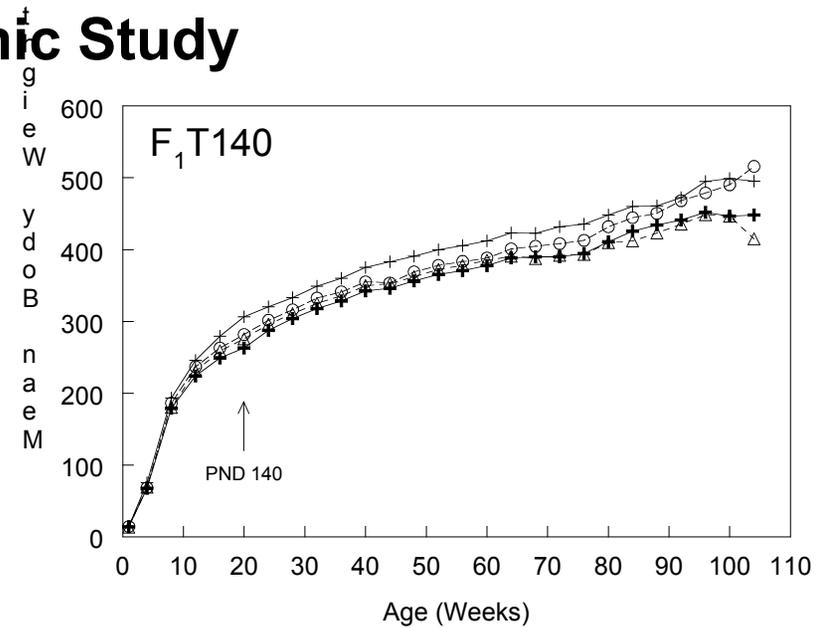
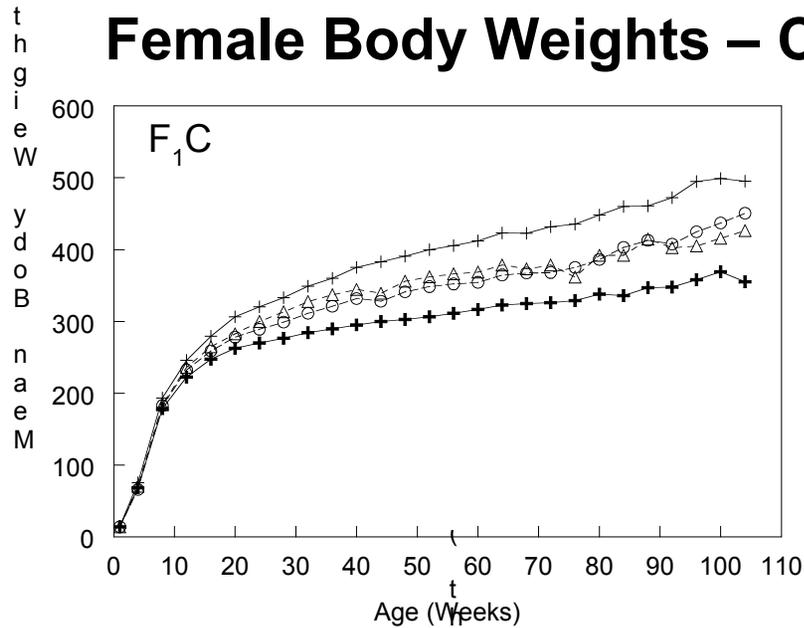
## Multigeneration Dosing Scheme



\* F<sub>4</sub> generation was mated as F<sub>0</sub> to F<sub>3</sub> to produce F<sub>5</sub> litters

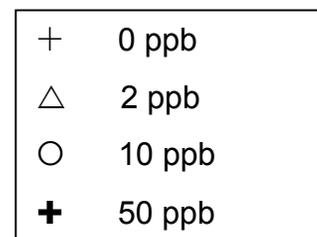
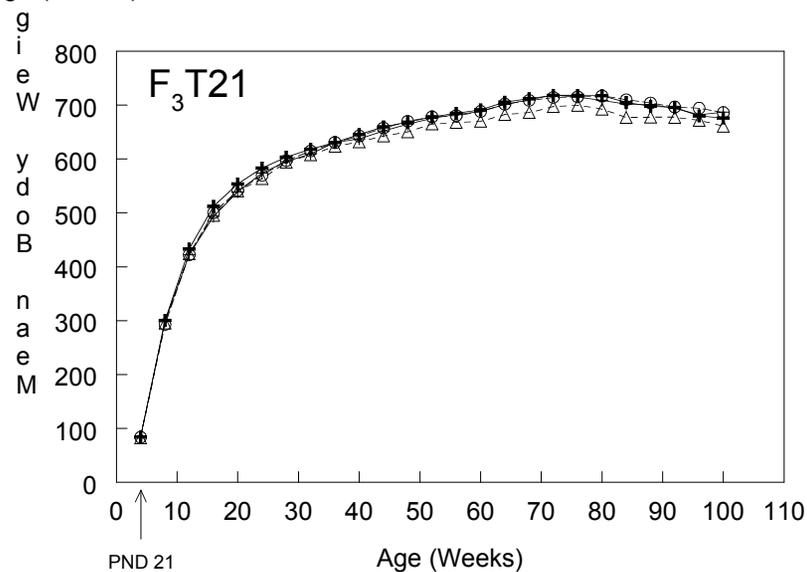
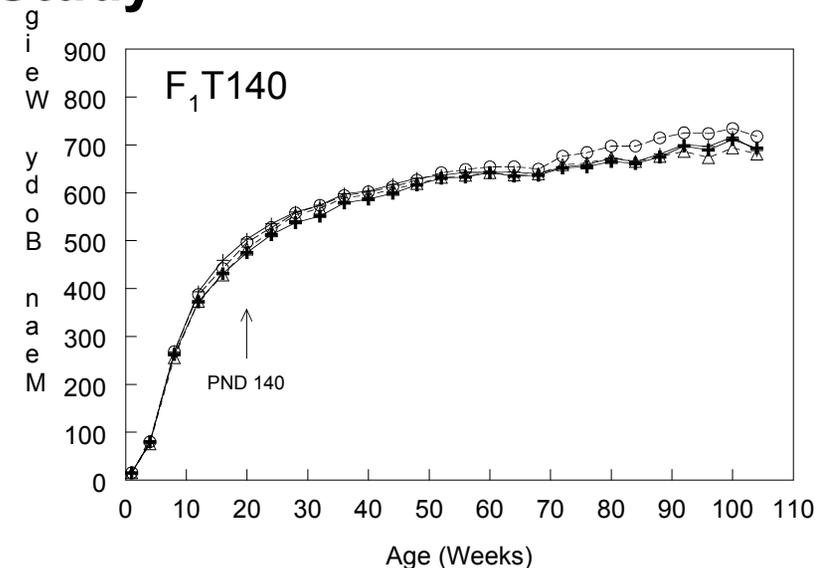
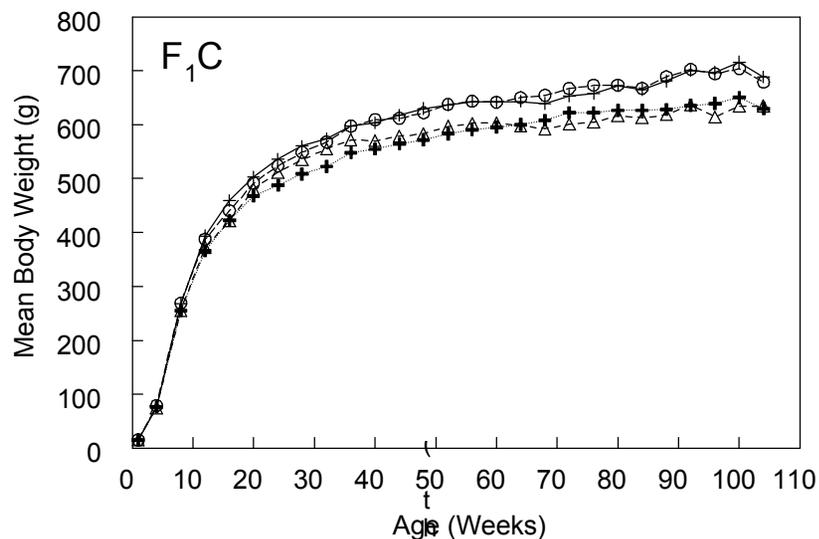


# Female Body Weights – Chronic Study





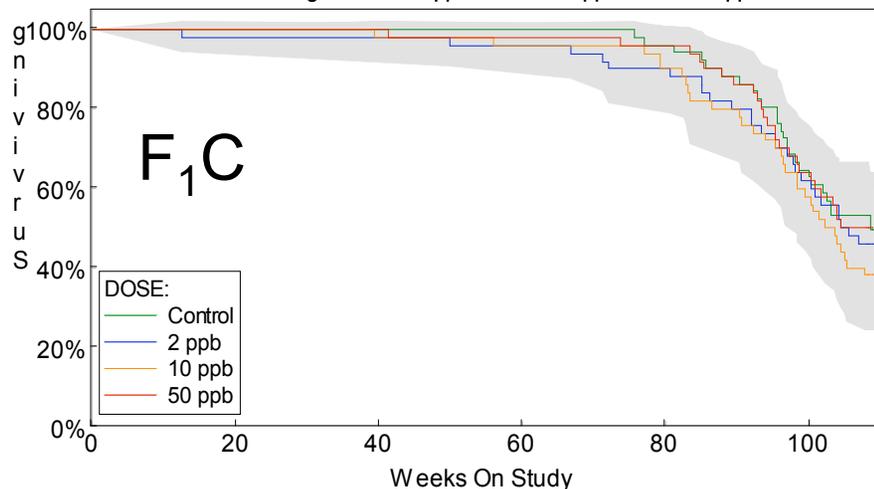
## Male Body Weights – Chronic Study



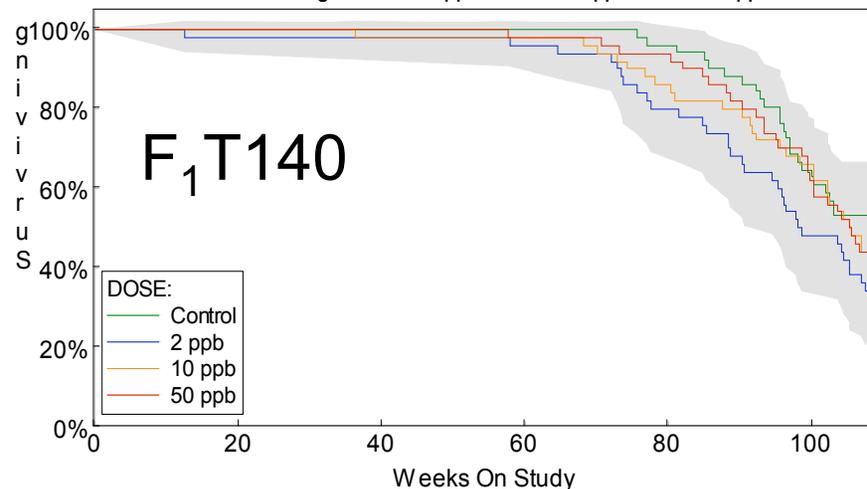


## Female Survival – Chronic Study

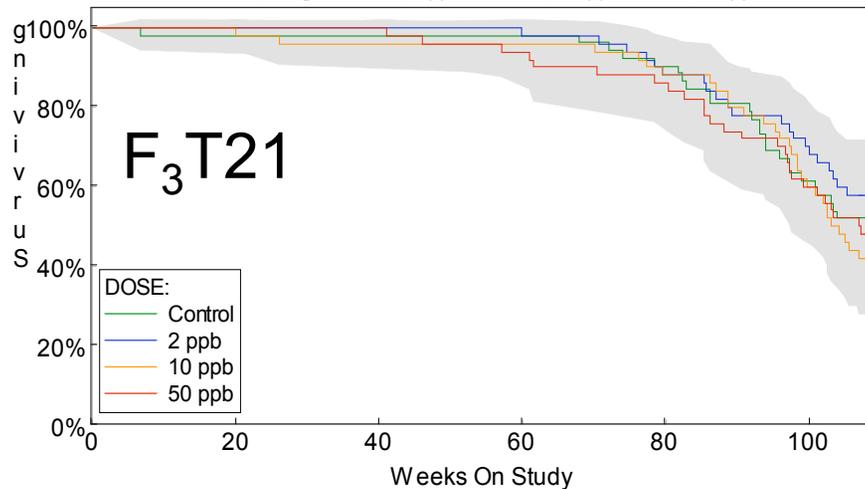
F1C - Linear P=0.370N, Log P=0.358, 2 ppb=0.277, 10 ppb=0.099, 50 ppb=0.441



F1T140 - Linear P=0.375N, Log P=0.419, 2 ppb=0.016, 10 ppb=0.232, 50 ppb=0.257



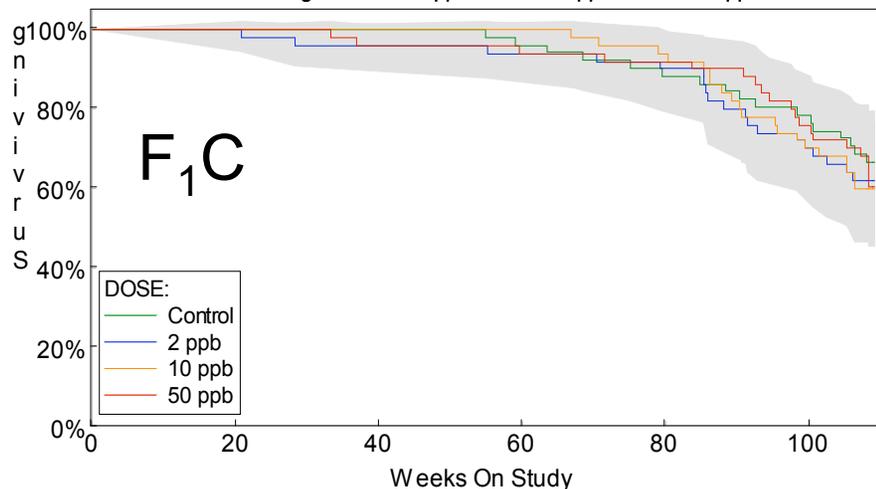
F3T21 - Linear P=0.253, Log P=0.206, 2 ppb=0.252N, 10 ppb=0.245, 50 ppb=0.343



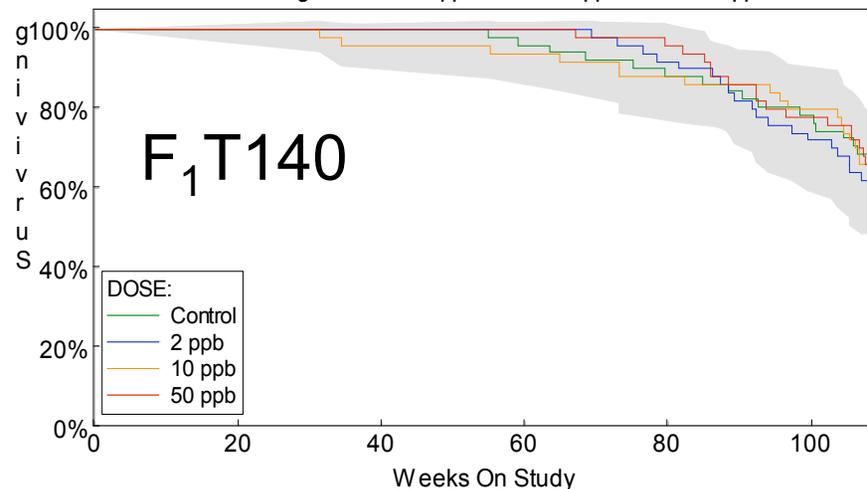


## Male Survival – Chronic Study

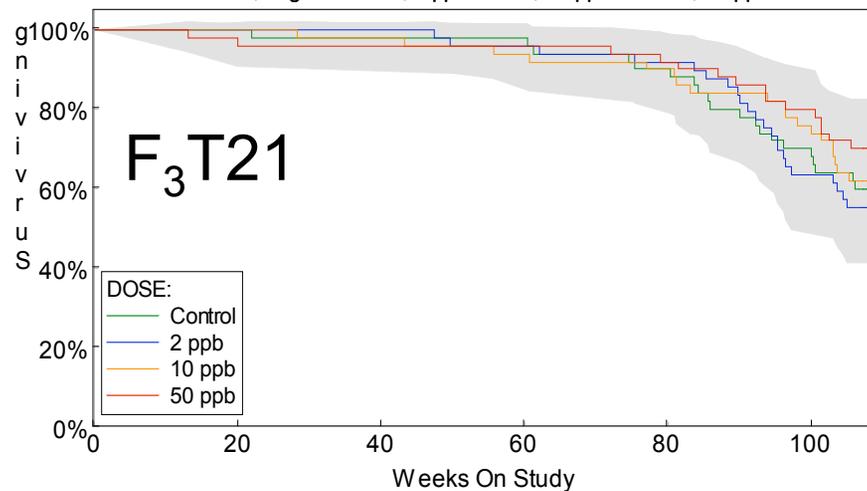
F1C - Linear P=0.449N, Log P=0.407, 2 ppb=0.294, 10 ppb=0.264, 50 ppb=0.395



F1T140 - Linear P=0.395N, Log P=0.433N, 2 ppb=0.326, 10 ppb=0.481, 50 ppb=0.486N



F3T21 - Linear P=0.079N, Log P=0.105N, 2 ppb=0.353, 10 ppb=0.386N, 50 ppb=0.134N



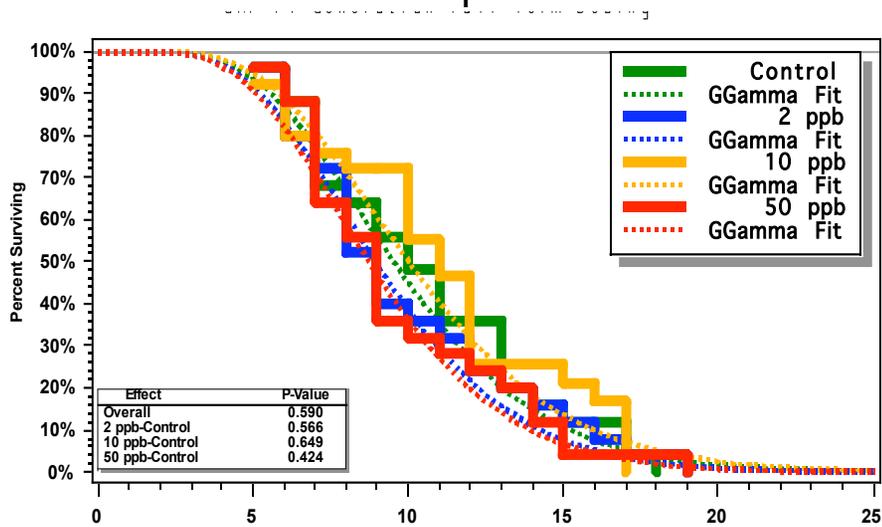


## **Vaginal Cytology -Chronic Phase**

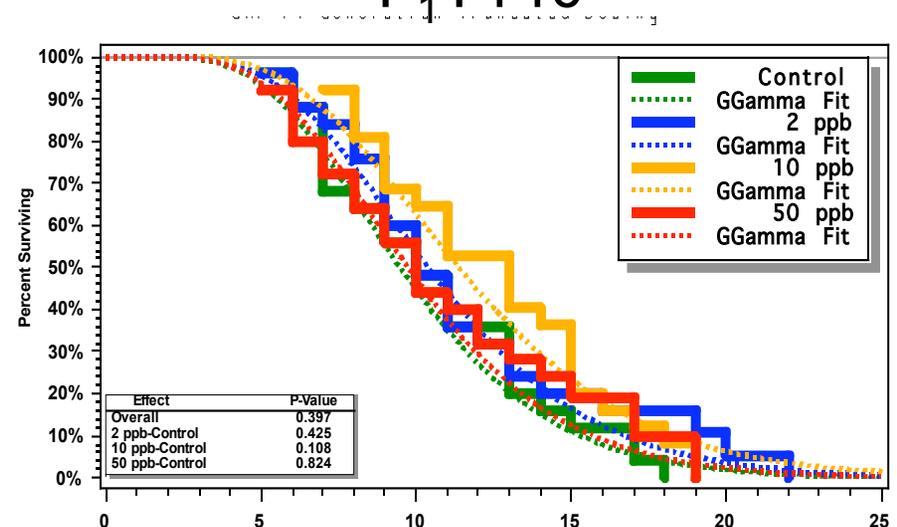
- Starting at 5 months, lavage a subset (25) of females from each dose group in each generation for 5 consecutive days once a month
- Normal cycle is 4-5 days
- Animals not showing cyclicity for 2 months in a row are considered to have aberrant cycles and are not sampled again



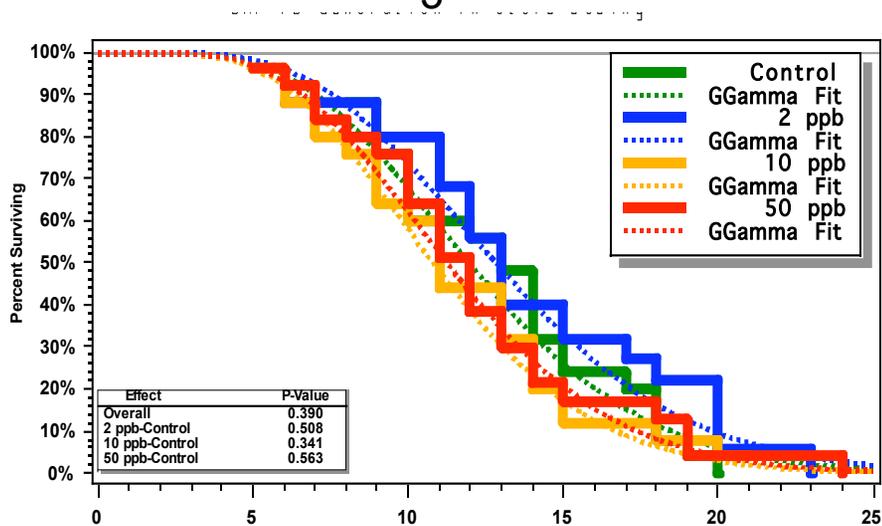
### F<sub>1</sub>C



### F<sub>1</sub>T140



### F<sub>3</sub>T21



No effect of treatment in any study arm on onset of aberrant cycles



## Males – Nonneoplastic Lesions

<b>Lesion</b>	<b>F<sub>1</sub>C</b>	<b>F<sub>1</sub>T140</b>	<b>F<sub>3</sub>T21</b>
Mammary gland, alveolar hyperplasia	1/44, 4/45, <b><u>6/47</u></b> , <b><u>18/44</u></b>	1/44, 2/45, <b><u>6/47</u></b> , <b><u>14/48</u></b>	2/42, 6/42, 4/40, <b><u>9/45</u></b>
Mammary gland, ductal hyperplasia	0/44, 0/45, 2/47, <b><u>3/44</u></b>	0/44, 0/45, 1/47, <b><u>3/48</u></b>	-
Liver, basophilic focus	1/49, 3/49, 3/50, <b><u>17/49</u></b>	1/49, 3/50, <b><u>11/48</u></b> , <b><u>6/49</u></b>	-
Liver, eosinophilic focus	3/49, 5/49, 8/50, <b><u>15/49</u></b>	3/49, 11/50, 5/48, <b><u>10/49</u></b>	-
Preputial gland, atrophy	0/51, 2/50, <b><u>5/50</u></b> , <b><u>4/50</u></b>	0/51, <b><u>6/50</u></b> , <b><u>3/50</u></b> , <b><u>6/50</u></b>	-

“-” = no significant effects; bolded, underlined text = significantly different from control



## Neoplasms - Males

Lesion	F <sub>1</sub> C	F <sub>1</sub> T140	F <sub>3</sub> T21
<b>Preputial gland</b>			
Squamous cell carcinoma	-	-	<b><u>2/49</u></b> , 3/49, 4/50, 7/49
Carcinoma	-	-	0/49, 1/49, 0/50, 1/49
Combined epithelial neoplasms	-	-	<b>2/49*</b> , 4/49, 4/50, <b><u>8/49</u></b>
<b>Mammary gland</b>			
Adenoma/adenocarcinoma	-	-	<b>0/42*</b> , 0/42, 0/40, 3/45

“-” = no significant effects; “\*” = significant increasing trend; underlined text= marginal trend (p <0.10) or difference from control



## Females – Nonneoplastic Lesions

<b>Lesion</b>	<b>F<sub>1</sub>C</b>	<b>F<sub>1</sub>T140</b>	<b>F<sub>3</sub>T21</b>
Uterus, endometrial hyperplasia	17/51, 18/50, 22/49, <b><u>25/50</u></b>	-	-
Uterus, squamous metaplasia	2/51, 6/50, <b><u>8/49</u></b> , <b><u>13/50</u></b>	-	1/52, 4/50, 3/50, <b><u>11/50</u></b>
Uterus, atypical focal hyperplasia	6/51, <b><u>14/50</u></b> , <b><u>16/49</u></b> , <b><u>20/50</u></b>	-	6/52, <b><u>16/50</u></b> , <b><u>15/50</u></b> , <b><u>21/50</u></b>
Clitoral gland, hyperplasia	-	2/50, 1/50, 2/49, <b><u>8/49</u></b>	0/50, 1/50, 2/49, <b><u>3/48</u></b>
Liver, basophilic focus	-	1/51, 1/50, 1/50, <b><u>6/50</u></b>	-
Liver, eosinophilic focus	1/51, 1/50, 1/49, <b><u>5/50</u></b>	-	-

“-” = no significant effects; bolded, underlined text = significantly different from control



## Neoplasms - Females

Lesion	F <sub>1</sub> C	F <sub>1</sub> T140	F <sub>3</sub> T21
Uterus			
Stromal polyps	-	<b>2/51*</b> , 5/50, 6/50, 7/50	1/52, <b><u>7/50</u></b> , 2/50, 5/50

“-” = no significant effects; “\*”, significant increasing trend;  
bolded, underlined text = significantly different from control



## Other Lesions – Female Mammary Gland

- Nonneoplastic
  - F<sub>1</sub>C: Galactocele, increasing trend (2/51, 2/49, 4/49, 6/50)
  - F<sub>3</sub>T21: Atypical focus (12/52, **21/50**, **22/50**, **18/50**)
- Neoplastic
  - F<sub>3</sub>T21: Adenocarcinoma, marginal increasing trend (6/52, 6/50, 9/50, 10/50)  
  
Fibroadenoma, marginal increasing trend (36/52, 34/50, 40/50, 37/50)



## TR 548 - Summary of Lesions

		Males	Females
F <sub>1</sub> C	Nonneoplastic	Mammary gland Liver	Uterus Liver
	Neoplastic	None	None
F <sub>1</sub> T140	Nonneoplastic	Mammary gland Liver	Clitoral gland Liver
	Neoplastic	None	Equivocal: uterine stromal polyps
F <sub>3</sub> T21	Nonneoplastic	Mammary gland	Uterus Clitoral gland
	Neoplastic	Some evidence: preputial gland	Equivocal: uterine stromal polyps



## Future Plans for the NTP Endocrine Disruptor Studies

- Comparison of the results of the 3 multigenerational reproductive studies (genistein, *p*-nonylphenol, ethinyl estradiol) and the 2 chronic studies (genistein and ethinyl estradiol) that were conducted under identical conditions
  - One report (*p*-nonylphenol) remains to be completed
- Interest in both common and discrepant effects and those showing evidence for carryover across generations
- Evaluate some features of the studies for inclusion in standard toxicity testing protocols