Draft NTP Technical Report TR585 on Green Tea Extract

Chad R. Blystone, PhD
National Institute of Environmental Health Sciences

NTP Technical Reports Peer Review Meeting
May 22, 2014

Nomination

- Epigallocatechin gallate (EGCG) nominated by National Cancer Institute
  - EGCG a component of Green Tea
  - Studied for therapeutic effects

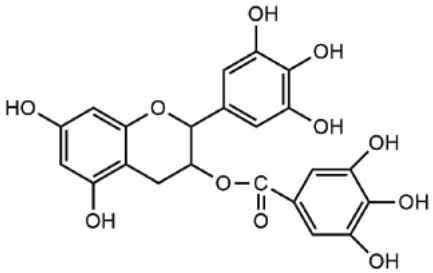
- NTP selected Green Tea Extract (GTE) for testing
  - More human exposure (multiple components)

Use and Exposure

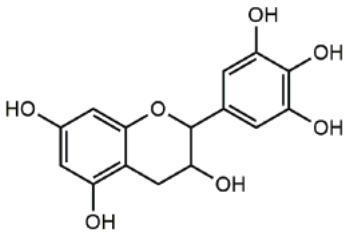
- GTE is a common herbal supplement
  - Reported (2008) fourth most common in use
  - Top 20 in sales in single or combination with other botanicals (2012)
- Supplements advertised for weight loss, anti-cancer, anti-heart disease, among other potential benefits.
  - Capsules: range in concentrations, alone or in combination with other botanicals
- Therapeutic potential is being studied
  - Either as GTE mixture or EGCG

GTE components

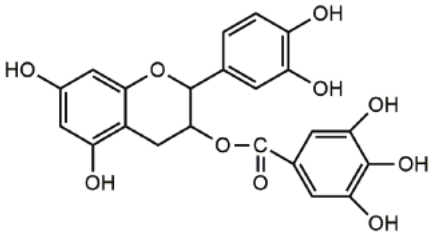
- Flavonoids and methylxanthines present
- Catechins are predominant flavonoids
- Four major catechins
  - EGCG, EGC, ECG, EC
- Preparations can vary widely in content



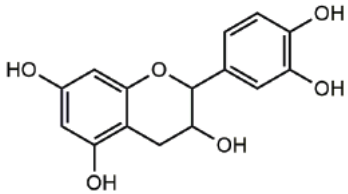
Epigallocatechin-3-gallate (EGCG)



Epigallocatechin (EGC)



Epicatechin-3-gallate (ECG)



Epicatechin (EC)

Test Article Selection

- Four lots of GTE from three sources analyzed for composition

Component	Range of four lots
Catechins	62.7 - 83.5%
EGCG	15.4 – 50.9%
	(15.4, 44.0, 46.9, 50.9)

- NTP selected a source based on:
  - EGCG Concentration
  - Similarity to other products
  - Availability in bulk quantity

Component	NTP Lot
EGCG	48.4%
ECG	12.8%
EGC	2.26%
EC	2.83%
Caffeine	4.99%

Study Design Overview for GTE

- Gavage (vehicle: deionized water): relevant to human exposure
- Rat gavage studies (male and female)
  - F344/NTac 3-month study: 0, 62.5, 125, 250, 500, 1000 mg/kg
  - Wistar Han 2-Year study: 0, 100, 300, 1000 mg/kg
    - Interim necropsy of 0 and 1000 mg/kg groups at 3-months
- Mouse gavage studies (male and female)
  - B6C3F1/N 3-month study: 0, 62.5, 125, 250, 500, 1000 mg/kg
  - B6C3F1/N 2-year study: 0, 30, 100, 300 mg/kg
- In vivo and in vitro genetic toxicity

GTE Genetic Toxicity

- *Salmonella typhimurium* TA98 and TA100
  - Mutagenic in presence of S9
  - Not mutagenic without S9 up to 2.0 mg/plate
- *Escherichia coli* WP2 uvrA/pkM101
  - Not mutagenic with or without S9 up to 1.0 mg/plate
- In vivo: B6C3F1/N mice 3-month study
  - No increase in micronucleated normochromatic erythrocytes up to 1000 mg/kg/day

F344/NTac 3-Month Studies

- Gavage five days/week (n=10): 0, 62.5, 125, 250, 500, 1000 mg/kg
- No effect on survival, some decrease in body weight: 14% (males) and 7% (females) decrease in 1000 mg/kg group compared to controls
- Decreased testis, lung (male), spleen (male), thymus (male and female) weights
  - Decreased epididymis weights, negative trend of caudal sperm, increase in estrous cycle length
- Lesions of the liver (female), nose (male and female), and thymus (male and female) attributed to GTE exposure
  - 1-3 of 10 females in 1000 mg/kg group with liver lesions
  - Minimal to mild lesions in nose of male and female 500 and 1000 mg/kg groups
  - Thymic atrophy in male and female 1000 mg/kg group

Wistar Han Rat 2-Year Dose Selection Rationale

- Survival and body weight effects not considered dose limiting in F344/NTac
- Minimal to mild histopathology not considered dose limiting in F344/NTac
- Doses of 0, 100, 300, and 1000 mg/kg selected for Wistar Han rats
  - Interim necropsy included to compare Wistar Han to the F344/NTac rat

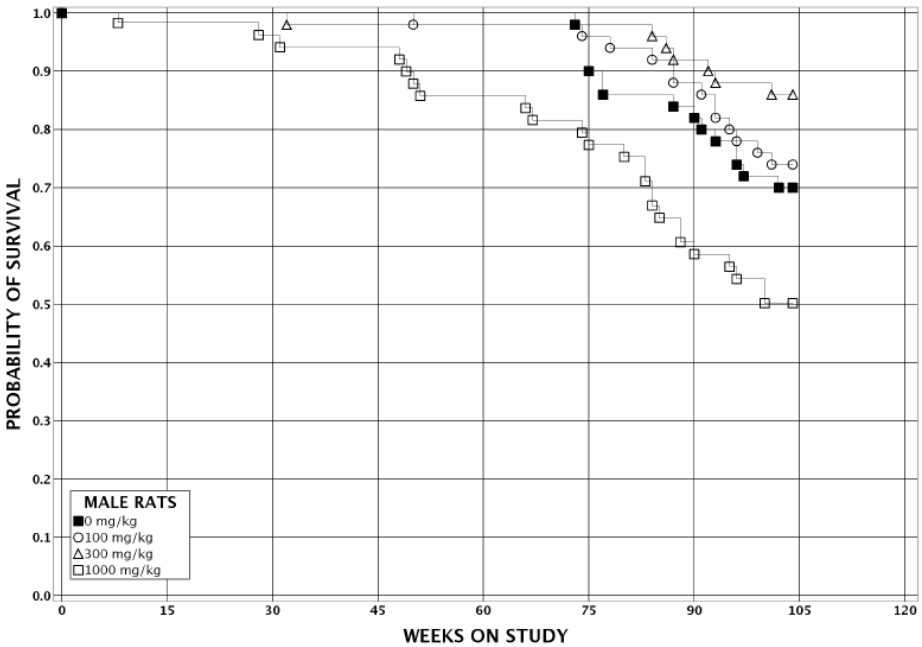
Wistar Han Rat - Interim Necropsy

Necropsy of 0 and 1000 mg/kg Wistar Han groups at 3-months (n=10)

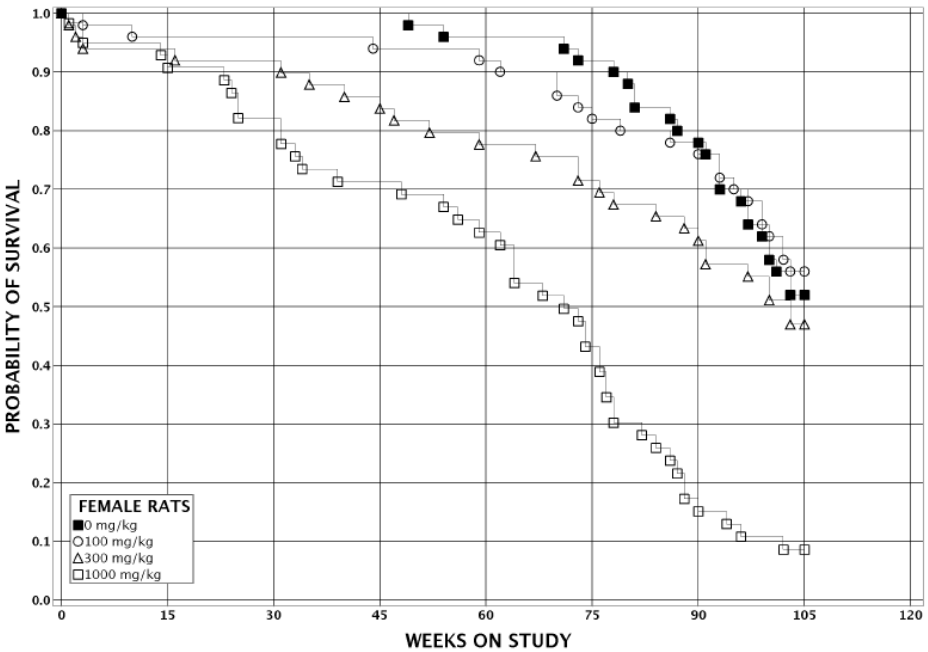
Endpoint	F344/NTac	Wistar Han
Body Weight[a]	Decrease (M & F)	No Effect
Organ Weights (decrease)[a]	Lung (M)	Thymus (F)
	Spleen (M)	
	Testis	
	Thymus (M & F)	
Histopathology	Liver (F)	Liver (F)
	Nose (M & F)	Nose (M & F)
	Thymus (M & F)	

[a] relative to concurrent control

Wistar Han Rat - Survival

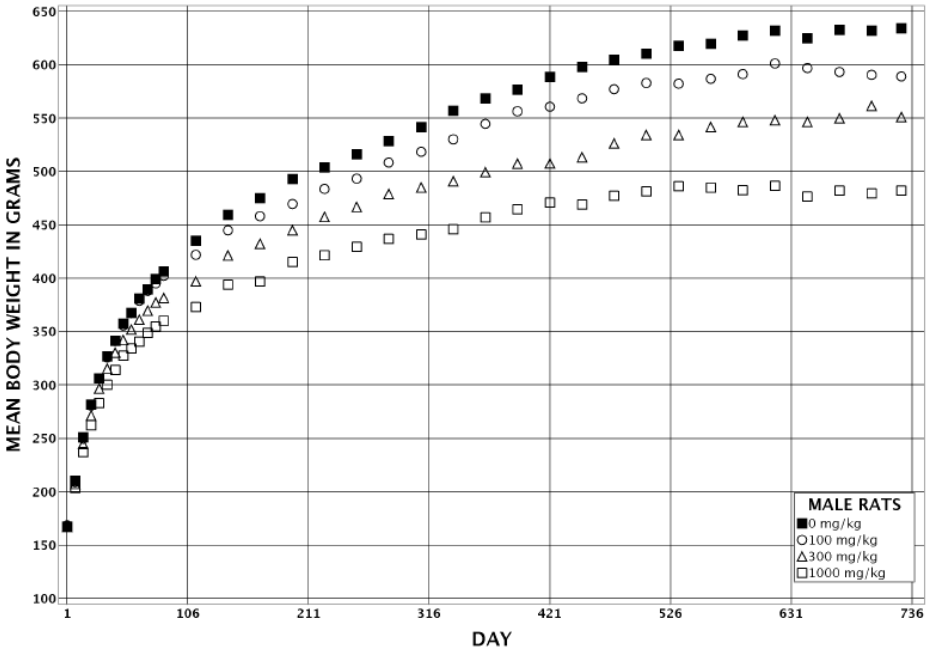


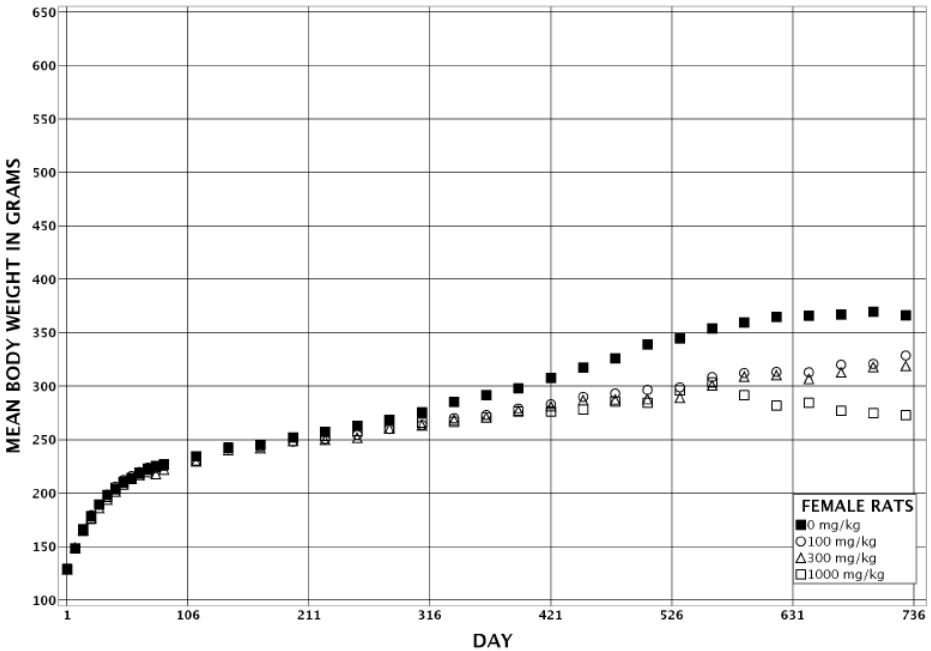
Decrease survival in 1000 mg/kg males



Decrease survival in 1000 mg/kg females

Wistar Han Rat - Body Weight





Male body weights reduced
100 mg/kg: up to 7%
300 mg/kg: up to 12%
1000 mg/kg: up to 24%

Female body weights reduced
100 mg/kg: up to 14%
300 mg/kg: up to 16%
1000 mg/kg: up to 26%

Wistar Han Rat – Pathology Summary

- No increase in tumor incidence attributed to GTE exposure

- Nonneoplastic lesions attributed to GTE exposure in males and females:
  - Liver
  - Gastrointestinal tract
  - Nose
  - Lung
  - Heart
  - Spleen
  - Bone marrow (female)

Wistar Han Rat - Liver Lesions

Liver	0 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
Male	50	50	50	50
Necrosis	1	2 (1.0)	2 (1.5)	13** (2.9)
Oval Cell Hyperplasia	0	0	0	2 (1.5)
Female	50	48	49	46
Necrosis	3 (2.7)	2 (2.0)	5 (2.6)	24** (3.3)
Oval Cell Hyperplasia	1 (1.0)	2 (1.0)	3 (1.7)	16** (1.3)

** $p \leq 0.01$
(Severity): 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Wistar Han Rat - Gastrointestinal Lesions

	0 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
Male				
Stomach Glandular, Mucosa, Necrosis	0/49	3/50 (2.0)	3/50 (1.7)	21/50** (2.5)
Intestine Small (Duodenum, Ileum, Jejunum), Mucosa, Necrosis	0/49	2/46	4/48	14/45**
Female				
Stomach Glandular, Mucosa, Necrosis	0/50	1/49 (2.0)	7/49** (1.1)	20/44** (1.9)
Intestine Small (Duodenum, Ileum, Jejunum), Mucosa, Necrosis	0/44	1/42	2/44	10/33**

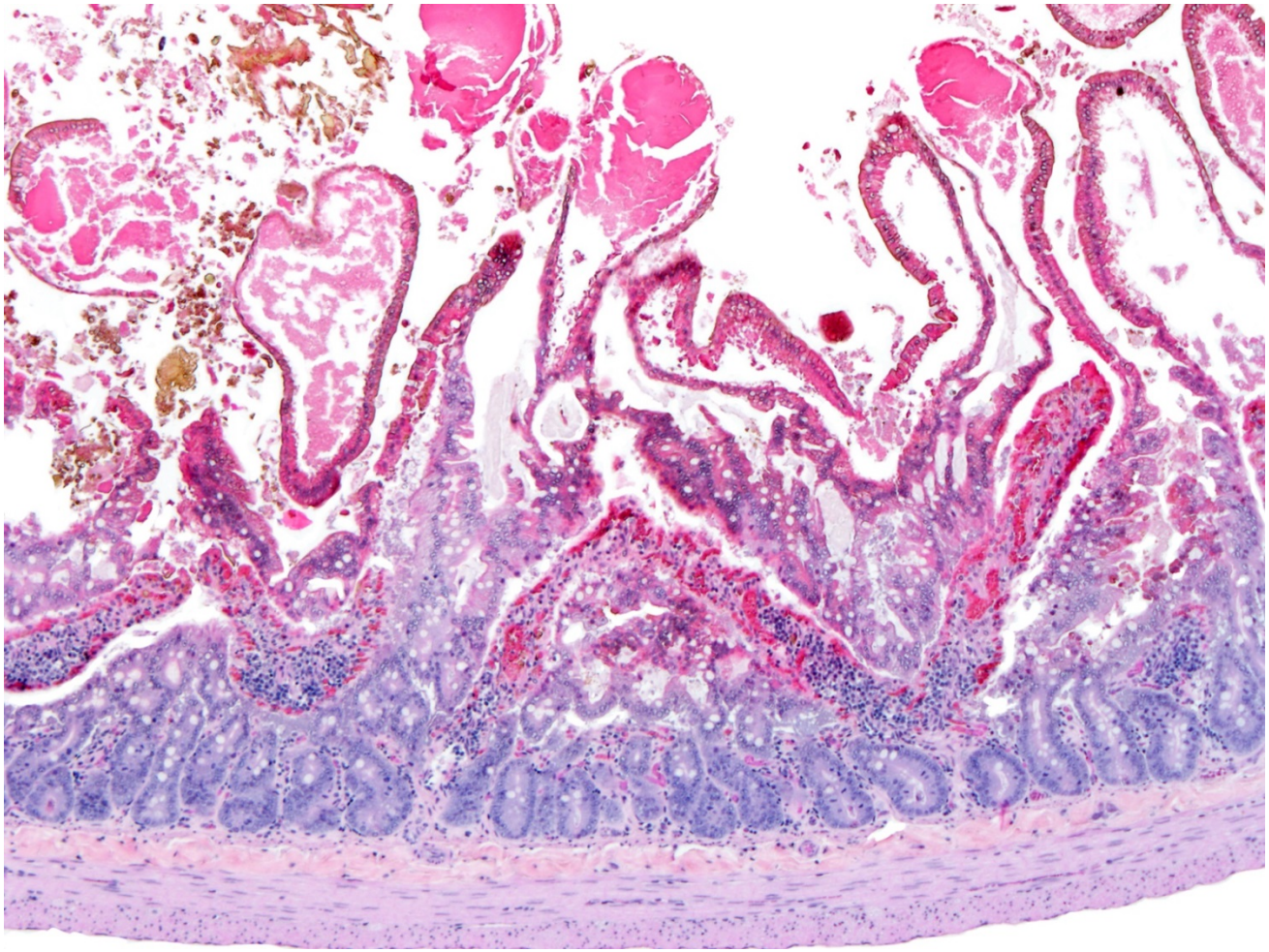
** $p \leq 0.01$
(Severity): 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Wistar Han Rat – Select Nose Lesions

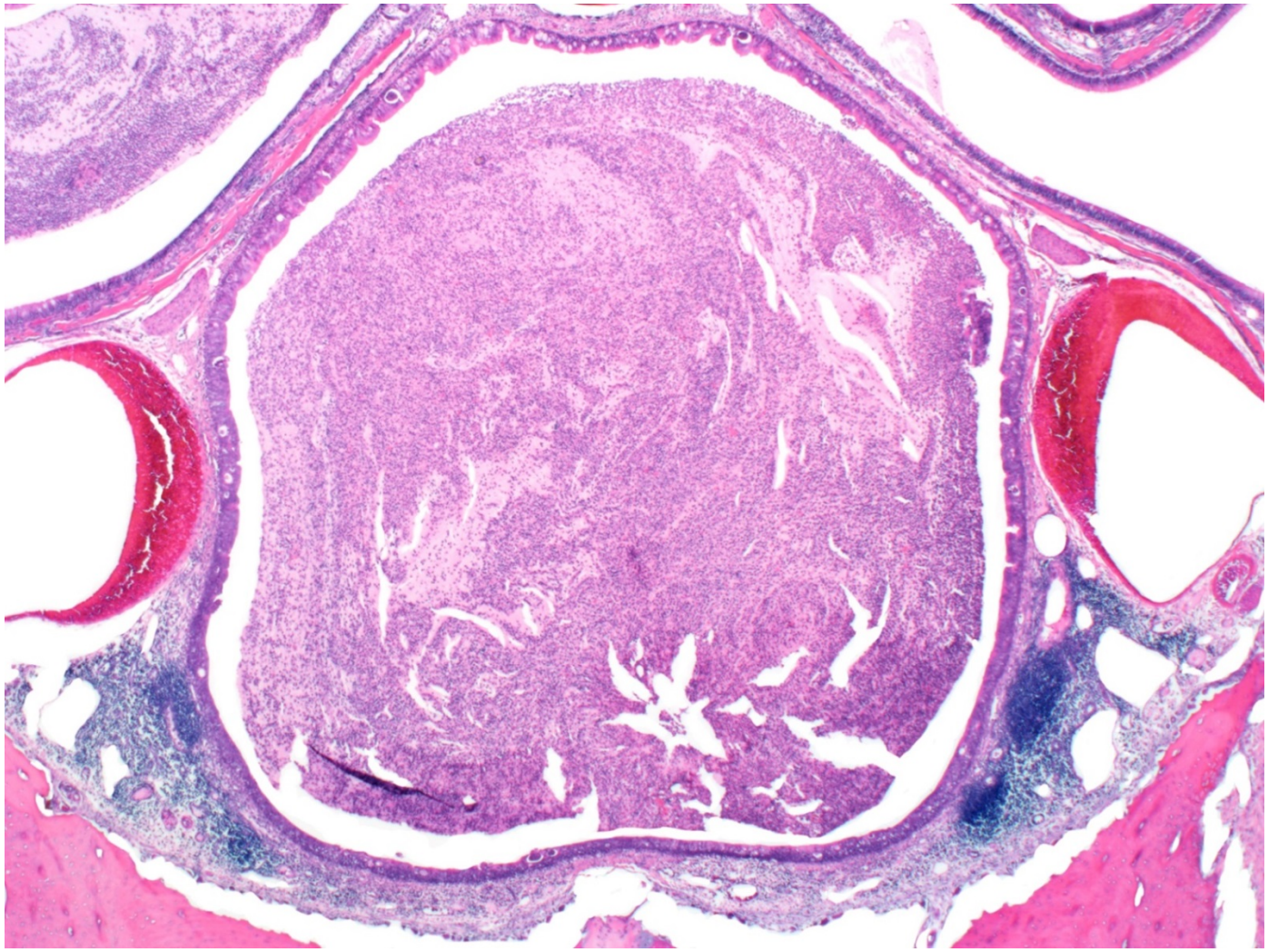
	0 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
Males	50	50	50	50
Inflammation, suppurative	11 (1.7)	12 (1.8)	20 (2.0)	42** (2.2)
Nerve, atrophy	0	33** (1.7)	44** (2.0)	44** (2.5)
Olfactory epithelium, atrophy	1	38** (1.8)	41** (2.0)	41** (2.0)
Olfactory epithelium, hyperplasia, basal cell	0	1 (1.0)	9** (1.0)	28** (1.8)
Olfactory epithelium, metaplasia, respiratory	4 (1.3)	40** (2.2)	43** (2.4)	47** (2.9)
Olfactory epithelium, necrosis	1 (2.0)	3 (2.0)	0	12** (2.0)
Turbinate, deformity	0	16**	22**	35**
Turbinate, hyperostosis	0	18** (1.5)	27** (1.9)	40** (2.2)

** $p \leq 0.01$
(Severity): 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

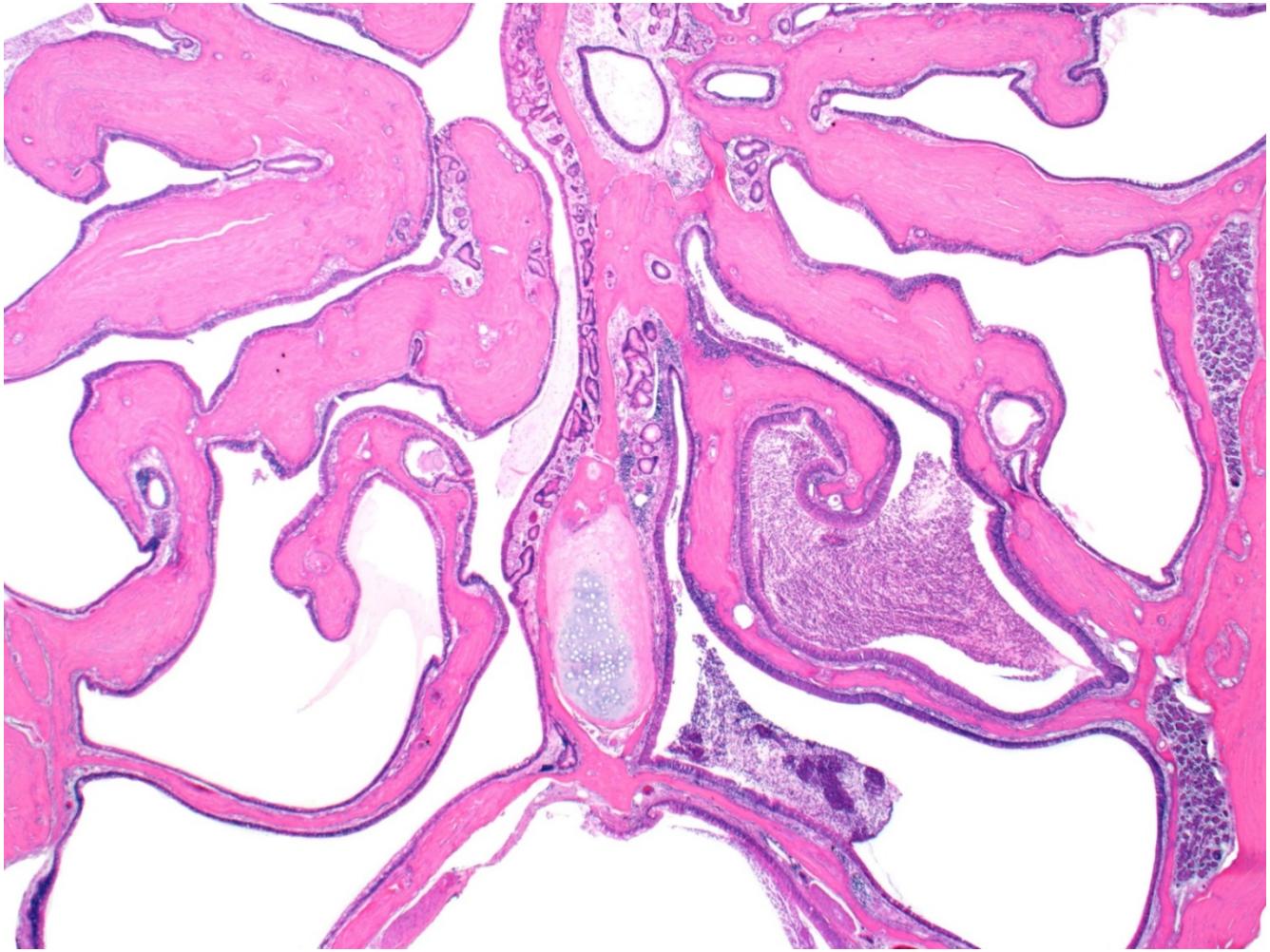
Gastrointestinal Pathology: 1000 mg/kg Male Rat



Nose Pathology: 1000 mg/kg Male Rat



Nose Pathology: 1000 mg/kg Male Rat



Wistar Han Rat - Lung, Spleen, Bone Marrow Lesions

	0 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
Male				
Lung, Suppurative Inflammation	0/50	1/50 (2.0)	3/50 (3.7)	10/50** (3.7)
Spleen, Lymphoid Depletion	1/50 (2.0)	2/50 (2.0)	1/50 (1.0)	13/50** (2.2)
Female				
Lung, Suppurative Inflammation	1/50 (2.0)	3/49 (2.0)	2/50 (3.5)	9/48** (3.4)
Spleen, Lymphoid Depletion	0/50	7/49** (1.7)	5/48* (1.8)	17/43** (1.7)
Bone Marrow, Hyperplasia	6/50 (1.7)	14/50* (2.1)	16/50* (2.4)	13/50* (2.5)

** $p \leq 0.01$
(Severity): 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

B6C3F1/N Mouse Studies of GTE

B6C3F1/N Mice 3-Month Studies

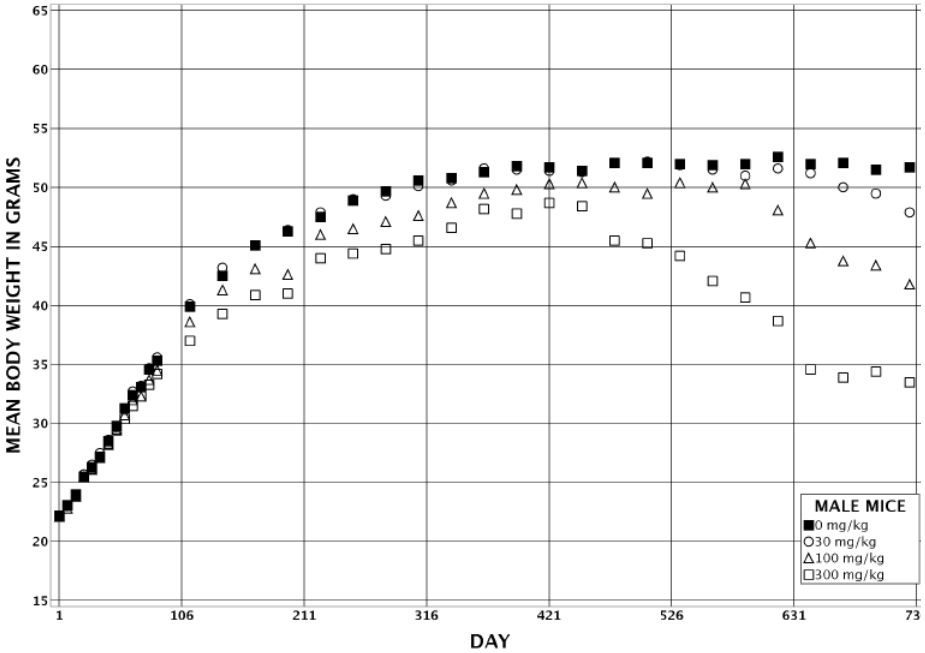
- Gavage five days/week (n=10): 0, 62.5, 125, 250, 500, 1000 mg/kg
- Decrease in male and female survival at 1000 mg/kg, decrease in body weight in males (up to 24% in 1000 mg/kg) and females (up to 14% in 1000 mg/kg) compared to control
- Decreased male and female thymus weights
- Decreased spermatid count and extended diestrus
- Lesions in male and female liver, nose, lymph node, thymus, intestine and spleen (female only) attributed to GTE exposure

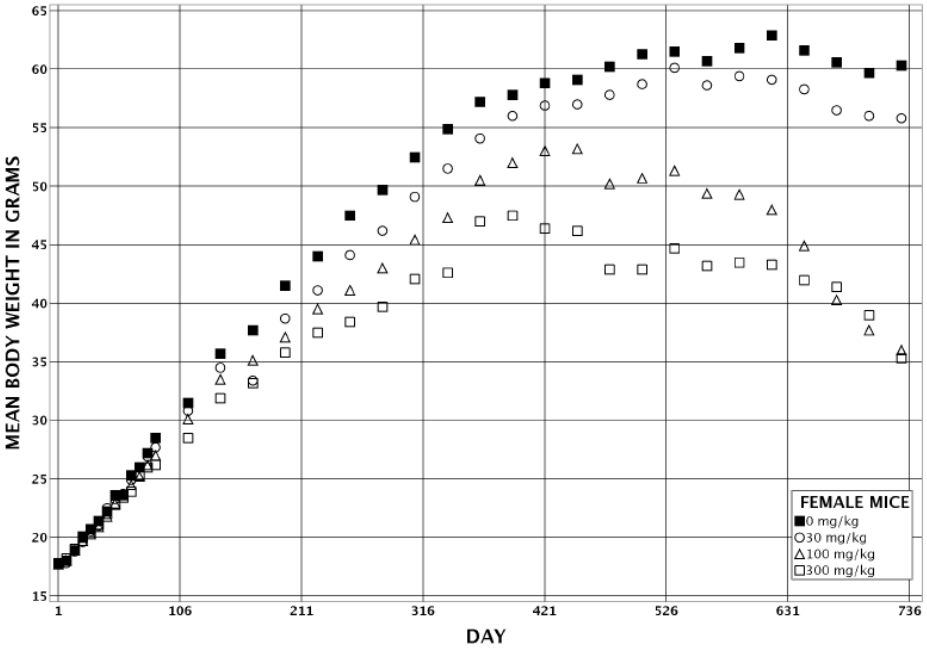
B6C3F1/N 2-Year Dose Selection Rationale

- Decreased survival in 1000 mg/kg group
- Decreases in body weight gain in 500 mg/kg group considered dose limiting
- Doses of 0, 30, 100, and 300 mg/kg selected for B6C3F1/N mice

B6C3F1/N Mice - Body Weight

No effects of GTE on male or female mice survival





Male body weights reduced
30 mg/kg: up to 4%
100 mg/kg: up to 16%
300 mg/kg: up to 35%

Female body weights reduced
30 mg/kg: up to 11%
100 mg/kg: up to 37%
300 mg/kg: up to 35%

B6C3F1/N Mice - Neoplastic Lesions

- No increase in tumor incidence attributed to GTE exposure in male mice

- Occurrence of papilloma and carcinoma of female mice tongue may have been related to GTE exposure

Tongue	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Females	50	47	48	45
Squamous cell papilloma	0	0	0	1
Squamous cell carcinoma	0	0	0	1
SC papilloma or carcinoma	0	0	0	2

Historical Control: 1/950 for papilloma; 0/950 for carcinoma

B6C3F1/N – Select Nose Lesions

	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Males	50	50	50	50
Foreign Body	1	10**	16**	25**
Hyperostosis	0	0	28** (1.5)	46** (1.6)
Inflammation, suppurative	14 (1.0)	40** (1.3)	49** (2.6)	48** (2.8)
Nerve, atrophy	0	26** (1.5)	49** (2.9)	50** (3.5)
Olfactory epithelium, atrophy	4 (1.5)	24** (1.3)	28** (1.6)	3 (1.0)
Olfactory epithelium, fibrosis	0	4 (1.3)	37** (1.8)	43** (2.8)
Olfactory epithelium, metaplasia, respiratory	11 (1.1)	45** (1.5)	49** (3.2)	49** (4.0)
Respiratory epithelium, squamous metaplasia	0	14** (1.3)	39** (1.6)	46** (2.3)
Respiratory epithelium, necrosis	0	7** (1.0)	16** (1.3)	27** (1.7)
Septum, perforation	1	0	26**	37**
Turbinate, deformity	0	0	41** (1.3)	50** (2.8)

** $p \leq 0.01$
(Severity): 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

B6C3F1/N – Liver Lesions

	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Male	50	50	50	50
Hematopoietic cell proliferation	2 (1.0)	2 (1.5)	6 (1.2)	10* (1.0)
Inflammation	4 (1.0)	1 (3.0)	5 (2.8)	12* (1.2)

** $p \leq 0.01$
(Severity): 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

B6C3F1/N - Bone Marrow and Lymph Node Lesions

	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Male	50	50	50	50
Bone Marrow, Hyperplasia	5 (1.2)	42** (1.6)	38** (1.6)	46** (1.5)
Mandibular Lymph Node				
Hyperplasia, Lymphoid	0	1 (1.0)	31** (2.2)	37** (2.1)
Infiltration Cellular, Plasma Cell	1 (1.0)	1 (1.0)	24** (1.3)	41** (1.6)
Female	50	50	50	50
Bone Marrow, Hyperplasia	6 (2.2)	11 (1.9)	41** (2.4)	34** (1.9)
Mandibular Lymph Node				
Hyperplasia, Lymphoid	0	1 (2.0)	8** (1.4)	12** (1.4)
Infiltration Cellular, Plasma Cell	0	0	31** (1.3)	18** (1.3)

** $p \leq 0.01$
(Severity): 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Conclusions

- *No evidence of carcinogenic activity* of green tea extract in male or female Wistar Han rats administered 100, 300, or 1,000 mg/kg.

- *No evidence of carcinogenic activity* of green tea extract in male B6C3F1/N mice administered 30, 100, or 300 mg/kg.

- *Equivocal evidence of carcinogenic activity* of green tea extract in female B6C3F1/N mice based on occurrences of squamous cell neoplasms of the tongue.

Conclusions

- Nonneoplastic lesions:
  - Liver, glandular stomach, small intestine (duodenum, ileum, and jejunum), nose, lung, heart, and spleen in male and female rats; bone marrow of female rats
  - Nose, mandibular lymph node, and bone marrow of male and female mice; and the liver of male mice

Questions