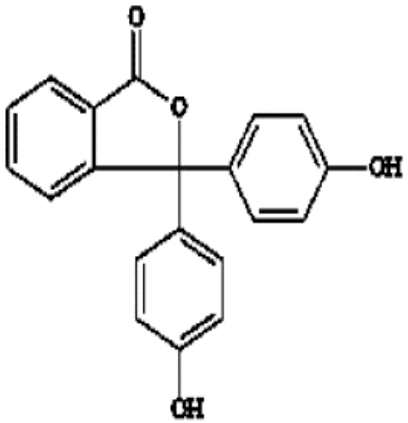
NTP
National Toxicology Program

Toxicology and Carcinogenesis Studies in Haploinsufficient p16^{Ink4a} /p19^{Arf} N2 Mice (B6.129-Cdkn2a^{tm1Rdp})

Phenolphthalein - GMM 12

Haploinsufficient p16^{Ink4a} /p19^{Arf} Mice Phenolphthalein Studies



Phenolphthalein

CAS No. 77-09-8

$C_{20}H_{14}0_4$

MW 318.33

- Used as laxative for most of the 20th century
- IARC- Group 2B- possible human carcinogen
- NTP ROC - reasonably anticipated to be a human carcinogen
- FDA ruling, 1999 - phenolphthalein removed as over-the-counter laxative

NTP Studies: Phenolphthalein Multisite Carcinogen

Phenolphthalein TR 465 (feed) - F344 rat and B6C3F1 Mouse

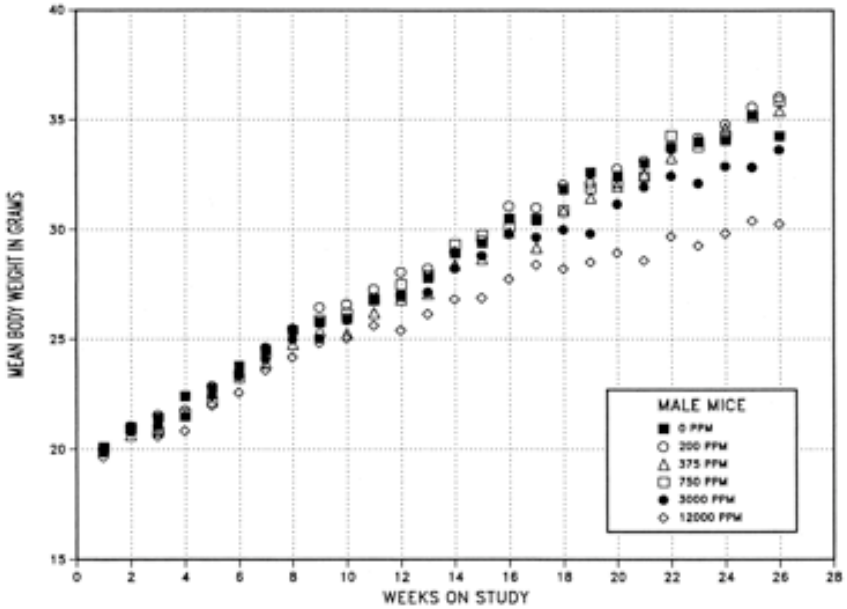
Male Rat	Female Rat	Male Mouse	Female Mouse
Adrenal medulla	Adrenal medulla	Histiocytic sarcoma	Histiocytic sarcoma
Kidney	Lymphoma	Lymphoma	
		Ovary	

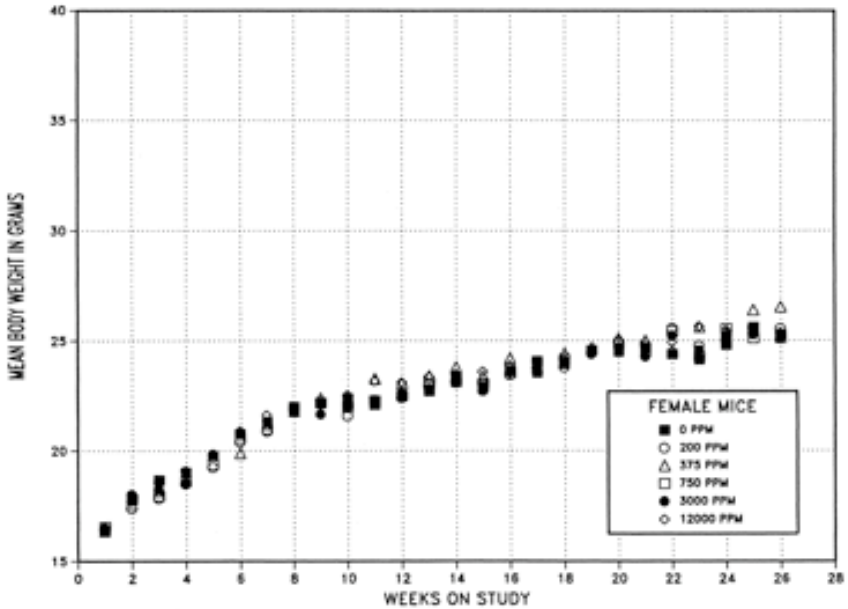
Positive in female p53(+/-) mice - thymic lymphoma (feed study)

Phenolphthalein Dose Selection for studies in Haploinsufficient p16^{Ink4a} /p19^{Arf} Mouse

- Doses selected to overlap NIEHS/NTP phenolphthalein doses
  - NTP 2-year B6C3F1 mouse phenolphthalein study
    - 0, 3000, 6000, 12,000 ppm
  - NIEHS 26-week p53(-/+) mouse phenolphthalein study
    - 0, 200, 375, 750, 3000, 12,000 ppm
- NTP 27-week haploinsufficient p16^{Ink4a}/p19^{Arf} mouse phenolphthalein study
  - 0, 200, 375, 750, 3000, 12,000 ppm
  - 15 animals/dose/sex
  - Chemical administered in feed

Body Weight - Male and Female Mice





Phenolphthalein: Males - Non-neoplastic Lesions

Dose (ppm)	0	200	375	750	3000	12,000
Spleen: Hematopoietic cell proliferation	2/14	5/15	3/15	1/14	2/15	14/14**
Kidney Nephropathy	6/14	7/15	8/15	6/14	15/15**	14/15**
Renal Tubule Hypertrophy	0/14	0/15	0/15	10/14**	15/15**	14/15**
Thymus Atypical Hyperplasia	0/14	0/15	1/15	0/14	1/15	0/15

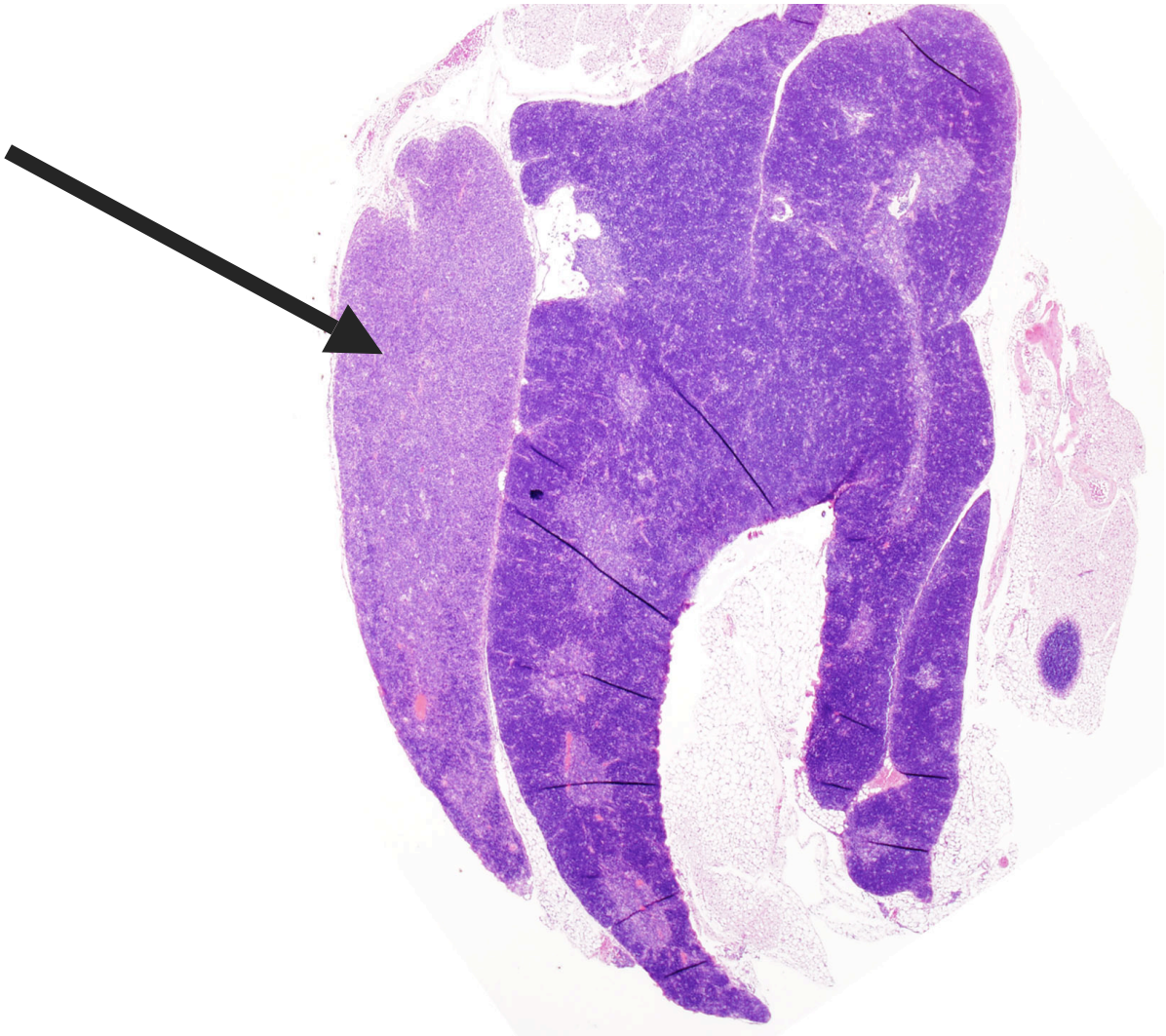
**$p \leq 0.01$

Phenolphthalein: Females - Non-neoplastic Lesions

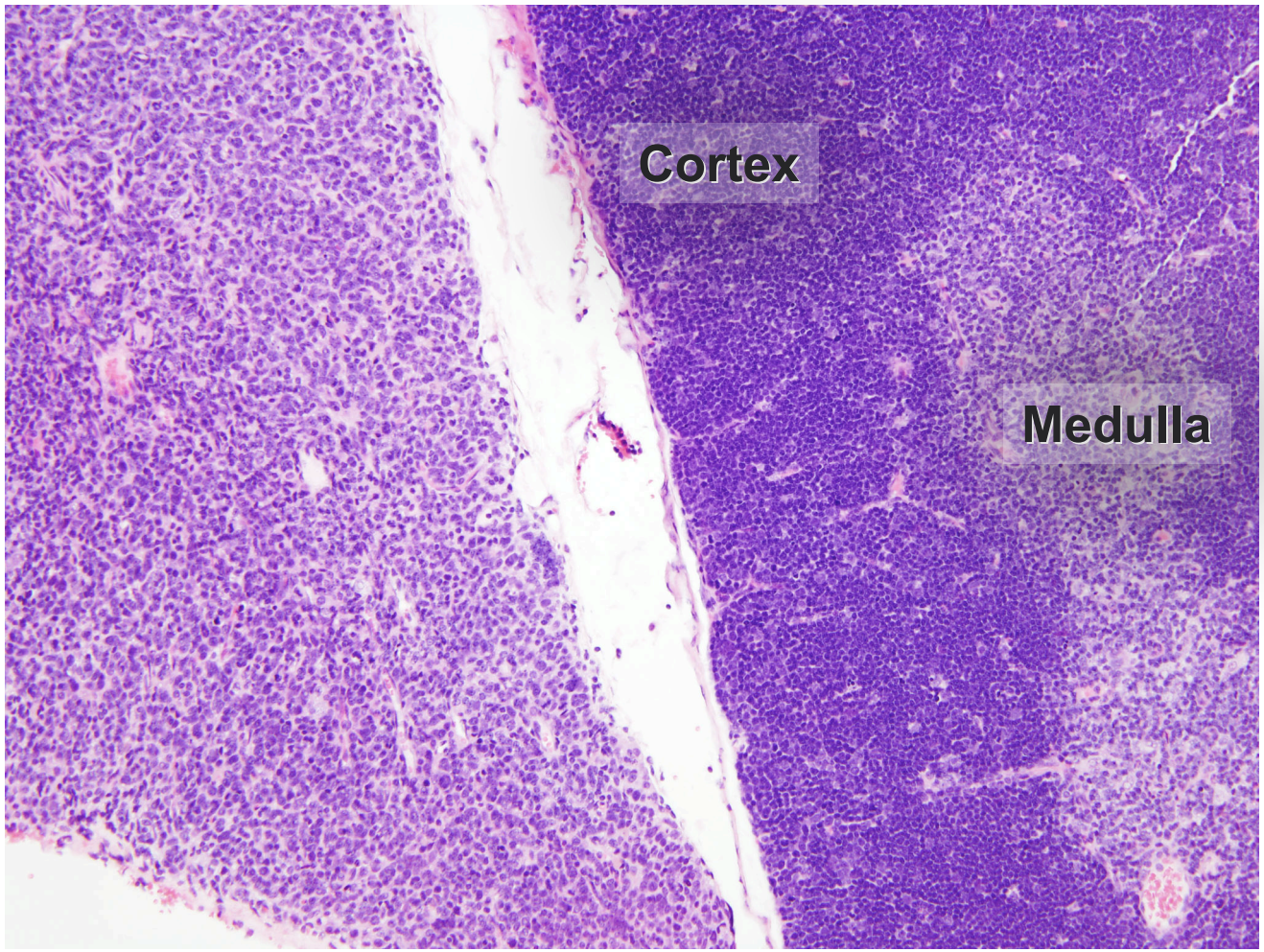
Dose (ppm)	0	200	375	750	3000	12,000
Spleen: Hematopoietic cell proliferation	2/15	5/14	9/14**	8/15*	7/15	13/15**
Thymus Atypical Hyperplasia	0/15	0/15	1/15	0/15	3/14	5/15*

* $p \leq 0.05$ ** $p \leq 0.01$

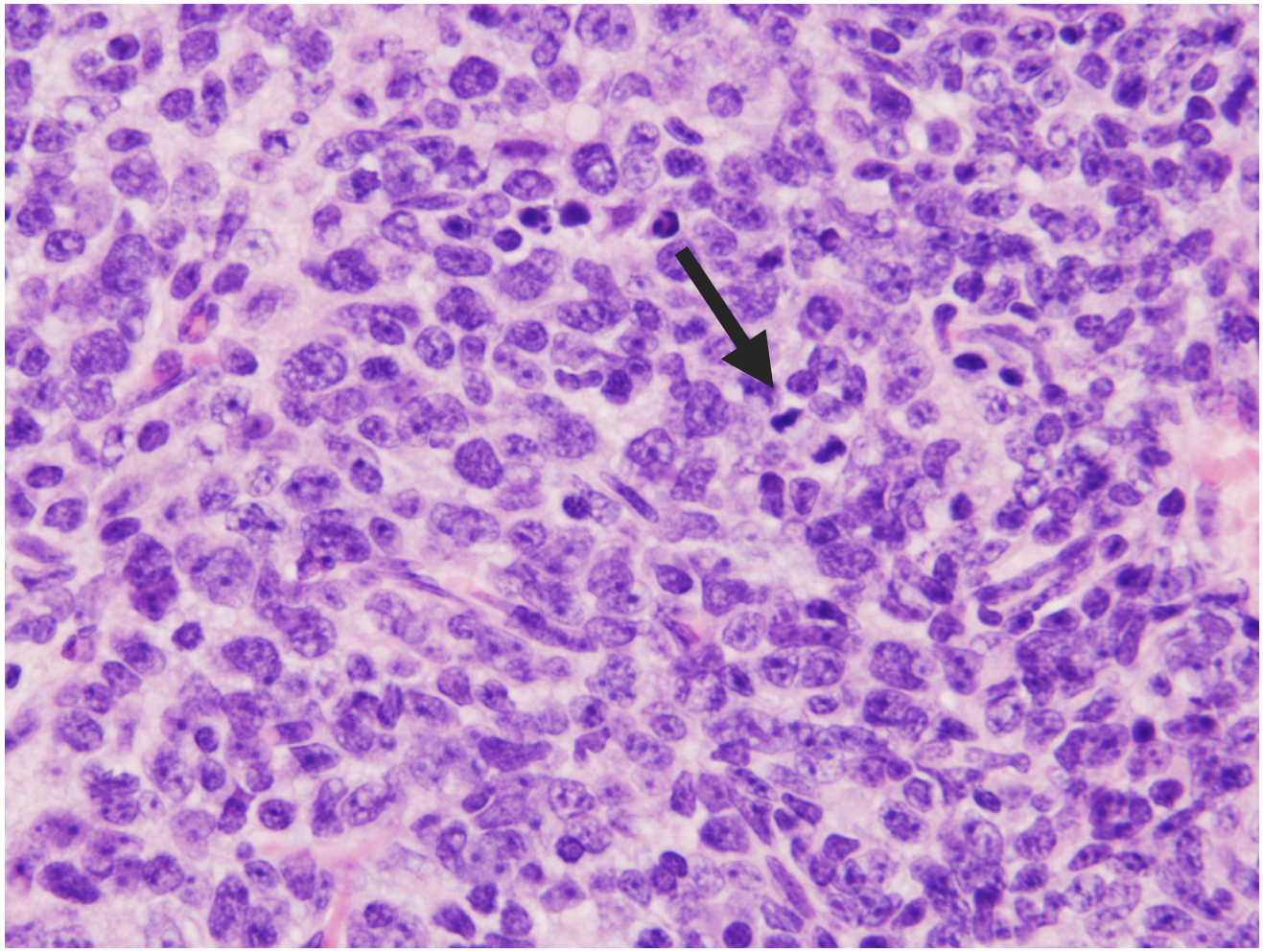
p16/p19 +/- Atypical Hyperplasia, Thymus



p16/p19 +/- Atypical Hyperplasia, Thymus



p16/p19 +/- Atypical Hyperplasia, Thymus



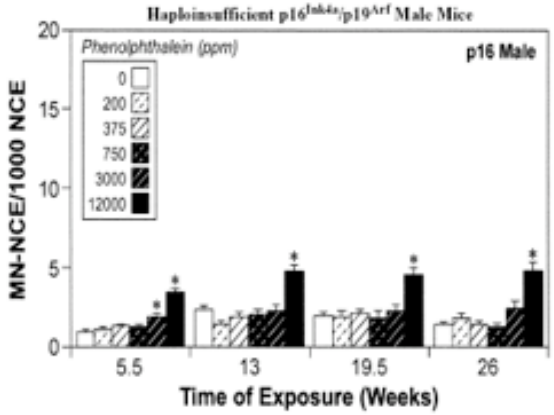
Phenolphthalein: Males - Reproductive Toxicity

Dose (ppm)	0	200	375	750	3000	12,000
Testis Atrophy, germinal epithelium	0/15	1/15	1/15	0/15	15/15**	14/15**
Interstitial cell (Leydig) Hyperplasia	0/15	1/15	1/15	0/15	15/15**	14/15**
Epididymis Hypospermia	0/15	0/15	1/15	0/15	15/15**	14/15**

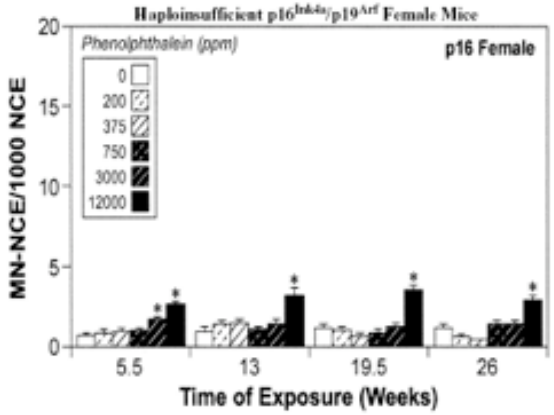
**$p \leq 0.01$

Phenolphthalein - Micronucleus Findings

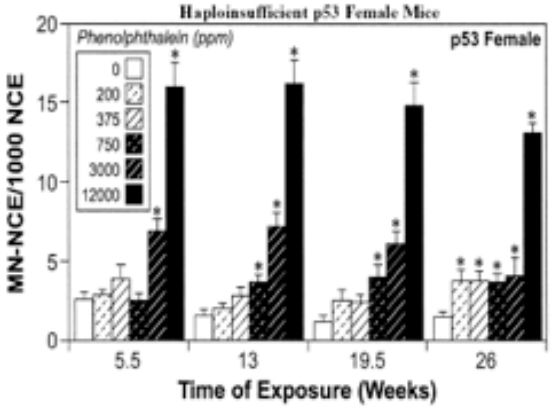
p16^{Ink4a} /p19^{Arf}
male mice



p16^{Ink4a} /p19^{Arf}
female mice



p53(+/-)
female mice



Phenolphthalein Conclusions:

- ***No evidence of carcinogenic activity*** of phenolphthalein in male or female haploinsufficient $p16^{Ink4a}$ /$p19^{Arf}$ mice exposed to 200, 375, 750, 3,000, or 12,000 ppm
- Treatment-related atypical hyperplasia, a preneoplastic lesion of the thymus, in mice
- Hematopoietic cell proliferation of the spleen in mice
- Kidney nephropathy and reproductive toxicity in male mice