NTP
National Toxicology Program

NTP TOXICOLOGY AND CARCINOGENESIS STUDIES OF A PENTABROMODIPHENYL ETHER MIXTURE [DE-71 (Technical Grade)]

(CAS NO. 32534-81-9)
(GAVAGE STUDIES)

NTP TR 589

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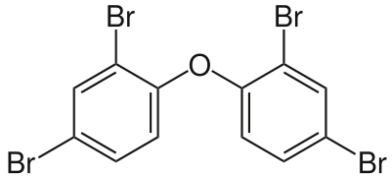
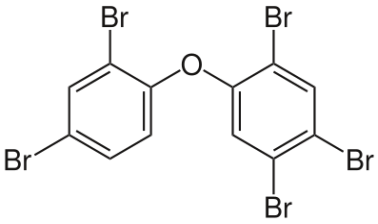
Outline of DE-71 Review Today

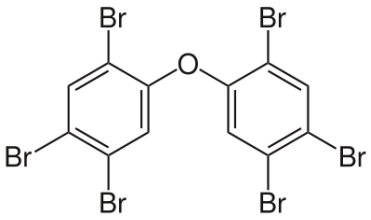
- DE-71 background
- Summary of selected findings in the NTP DE-71 studies
  - 3-month study results
  - 2-year study results
- Conclusions and level of carcinogenic activity



BDE-47



BDE-99



BDE-153

- Pentabromodiphenyl ethers nominated by California Office of Environmental Health Hazard Assessment (OEHHA)
- Pentabromodiphenyl ethers are flame retardants that are bioaccumulative and persistent organic pollutants
- DE-71 (technical grade), a mixture of PBDEs, was studied because it is what was produced and is representative of PBDE exposure to humans
- Widespread human exposure

PBDEs in DE-71

PBDE	Name	CAS #	% in DE-71
BDE-47	2,2',4,4'-Tetrabromodiphenyl ether	5436-43-1	35.68
BDE-100	2,2′,4,4′,6-Pentabromodiphenyl ether	189084-64-8	10.44
BDE-99	2,2′,4,4′,5-Pentabromodiphenyl ether	60348-60-9	41.67
BDE-85	2,2′,3,4,4′-Pentabromodiphenyl ether	182346-21-0	2.03
BDE-154	2,2′,4,4′,5,6′-Hexabromodiphenyl ether	207122-15-4	3.63
BDE-153	2,2′,4,4′,5,5′-Hexabromodiphenyl ether	68631-49-2	3.33

3-Month Study Design

- F344/N rats and B6C3F1/N mice (10 animals/sex/dose)
- oral gavage, corn oil, 5 days/wk, for 3 months
- 0, 0.01, 5, 50, 100, 500 mg/kg
- Additional rats added for 4 and 25 day endpoints (10/sex/dose)
- Treatment-related toxicity in Liver and Thyroid system
- BDE-47, BDE-99, BDE-153 measured in adipose & liver

DE-71, BDE-47, BDE-99, BDE-153:

- Negative in bacterial mutagenicity assays
- Negative in mouse erythrocyte micronucleus assay

DE-71 3-month study

F344/N Male Rat – selected liver findings

Dose (mg/kg)	0	0.01	5	50	100	500
Male Rat						
Final survival	10	10	10	10	10	10
Final Body wt (g)	316	335	327	330	318	272**
Liver wt (g)	10.09	11.22	12.13**	16.04**	17.42**	20.01**
Hepatocyte hypertrophy	0	0	9** (1.0)[a]	10** (2.7)	10** (3.4)	10** (3.7)
Hepatocyte vacuolization	0	0	0	10** (1.2)	10** (2.0)	10** (1.7)

*P<0.05; **P<0.01 N=10

[a]Severity grade (1 = minimal; 2 = mild; 3 = moderate; 4 = marked)

F344/N Rat – other lesions

Dose (mg/kg)	0	0.01	5	50	100	500
Male Rat						
Thyroid gland, follicle hypertrophy	0	0	0	0	1 (1.0)	9** (1.0)
Stomach, glandular erosion	0	0	1 (1.0)	2 (1.5)	3 (1.7)	4* (1.5)
Epididymis hypospermia	0	0	0	0	0	9** (1.9)
Female Rat						
Thyroid gland, follicle hypertrophy	0	0	0	8** (1.0)	9** (1.4)	10** (2.9)
Stomach, glandular erosion	0	0	0	0	0	3 (1.0)
Thymus atrophy	0	0	0	0	0	4* (1.3)

*P<0.05; **P<0.01 N=10

B6C3F1/N Male Mouse – selected liver findings

Dose (mg/kg)	0	0.01	5	50	100	500
Male Mice						
Final survival	10	10	10	10	10	3
Final Body wt (g)	39.3	38.8	39.3	37.3	35.9**	28.6**
Liver wt (g)	1.38	1.31	1.50	1.79**	2.18**	4.11**
Hepatocyte hypertrophy	0	0	1 (1.0)	10** (1.8)	10** (2.7)	10** (3.1)
Hepatocyte vacuolization	0	0	0	0	0	6** (1.2)
Hepatocyte focal necrosis	0	0	0	0	0	2 (2.0)
Hepatocyte necrosis	0	0	0	0	1 (1.0)	10** (1.3)

**P<0.01 N=10

B6C3F1/N Male Mouse – other lesions

Dose (mg/kg)	0	0.01	5	50	100	500
Male Mice						
Adrenal cortex degeneration fatty	0	0	0	0	0	4** (1.3)
Adrenal cortex, zona fasciculata hypertrophy	0	0	0	0	0	5** (1.0)
Testes abnormal residual body	0	0	1 (2.0)	0	1 (2.0)	5** (2.0)
Thymus atrophy	0	0	0	0	0	6** (2.5)

**P<0.01 N=10

B6C3F1/N Female Mouse – other lesions

Dose (mg/kg)	0	0.01	5	50	100	500
Female Mice						
Adrenal cortex degeneration fatty	0	0	0	0	0	2 (2.0)
Thymus atrophy	0	0	0	1 (2.0)	0	3 (3.3)

N=10

Clinical Chemistry, Hematology, Liver Enzymes – Selected Findings

- **Male & female rats (day 4, 25 and 93)**
  - **Thyroid effect:** ↓T4, ↑TSH
  - ↑serum cholesterol
  - ↓**Erythron:** ↓hematocrit, ↓hemoglobin, ↓MCH, ↓MCV
  - **Evidence of hemoconcentration**: ↑total protein, ↑albumin, ↑UN
  - **Liver (day 25, & 93):**
    - ↑PROD(CYP2B), ↑EROD(CYP1A1), ↑A4H(CYP1A2), ↑UDPG
    - ↑Bile salts, ↑SDH, ↑ALT

Clinical Chemistry, Hematology, Liver Enzymes – Selected Findings

- **Male & female mice (day 93)**
  - **Liver:**
    - ↑PROD(CYP2B), ↑EROD(CYP1A1), ↑A4H(CYP1A2), ↑UDPG
  - ↓**Erythron**: ↓hematocrit, ↓hemoglobin, ↓RBC

Reproductive toxicity endpoints

- Rats (0, 50, 100, 500 mg/kg)
  - ↓ Epididymis wt., sperm motility, sperm per cauda (primarily at 500 mg/kg)
  - No estrous cycling at 500 mg/kg
- Mice (0, 5, 50, 100 mg/kg)
  - ↓ Cauda Epididymis wt., sperm motility (at 100 mg/kg)

2-Year Study Design (oral gavage, corn oil)

- **Wistar Han dams [Crl:WI(Han)] Rat**
  - 0, 3, 15, 50 mg/kg
  - Time-mated dams: Dosing - GD6-PND20
  - Pups: Dosing - PND12-PND21
- **2-year rat dosing phase**
  - ~2 males and 2 females selected per litter assigned to 2 year dosing phase of the study on PND21
  - 50 animals/sex/dose from about 25-30 litters/dose group (core study)
  - Dosing 5X/wk: 0, 3, 15, 50 mg/kg
  - Interim evaluation at 3 months in 0 and 50 mg/kg (10/sex/grp)
- BDE-47, BDE-99, BDE-153 measured in adipose, liver, plasma

2-Year Study Design (oral gavage, corn oil)

- **B6C3F1/N Mouse** – adult exposure only
  - 0, 3, 30, 100 mg/kg
  - 50 animals/sex/dose
  - 5X/week

- BDE-47, BDE-99, BDE-153 measured in adipose, liver

Wistar Han Rat - Littering Parameters

- No significant treatment-related effects during perinatal exposure based on maternal, littering and pup parameters
  - # pups per litter, pup weights, dam weights, # dams with litters, # dams not pregnant, # of dams littering
- No litter effect on the occurrence of treatment-related tumors

Wistar Han Rat - Interim Sac at 3 months

Dose (mg/kg)	Males 0	Males 50	Females 0	Females 50
Mean body wt (g)	403	433	246	213**
Mean liver wt (g)	13.68	19.53**	7.94	9.28*
Hepatocyte hypertrophy	0/10	10/10** (3.1)	0/9	10/10** (3.0)
Hepatocyte fatty change	2/10 (1.0)	8/10** (1.5)	0/9 (1.0)	3/10 (1.0)
Thyroid gland follicle hypertrophy	0/10	4/10* (1.3)	1/10 (1.0)	5/10 (1.2)

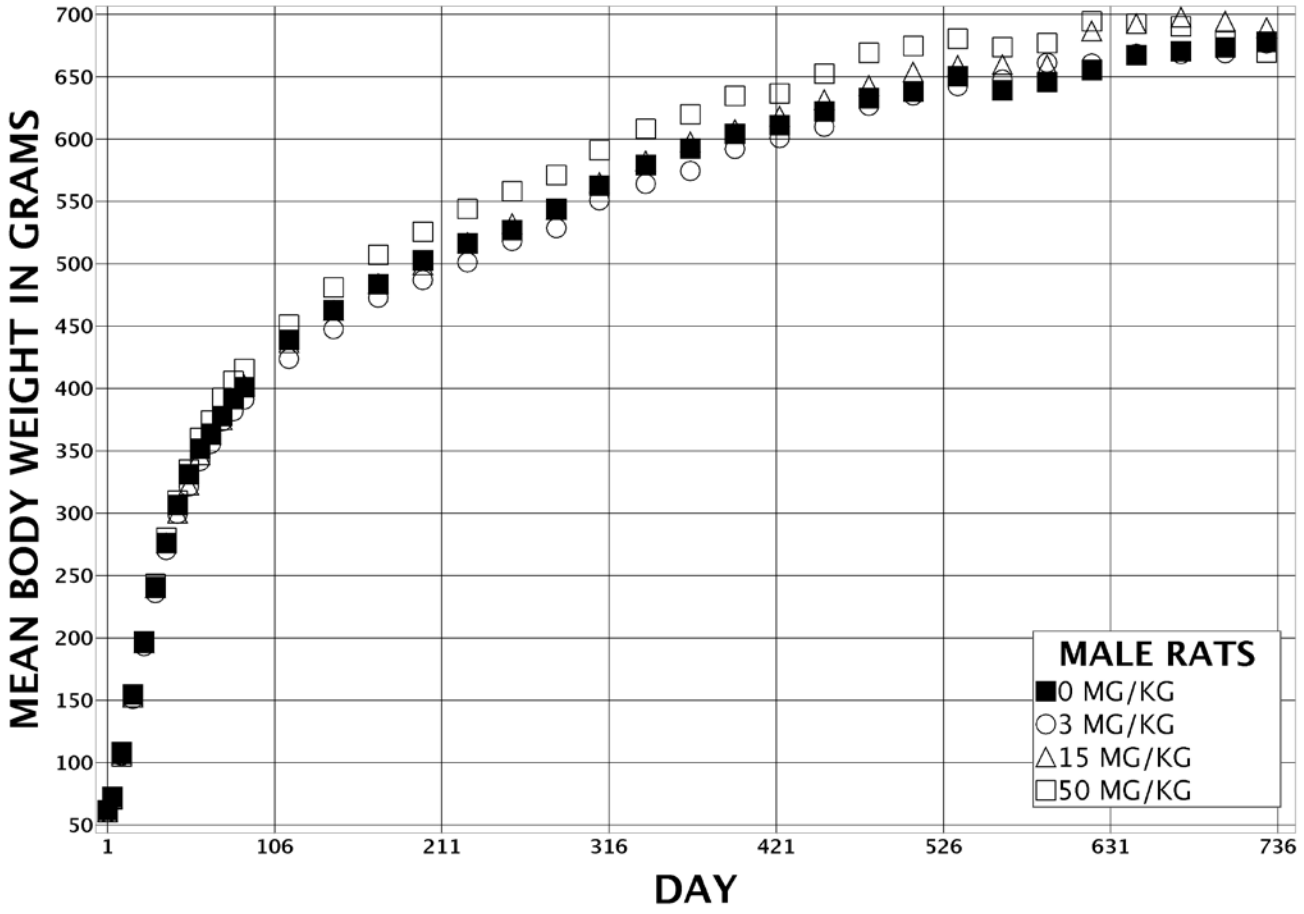
*P<0.05; **P<0.01

Wistar Han Rat - Final Survival & Mean Body Weight

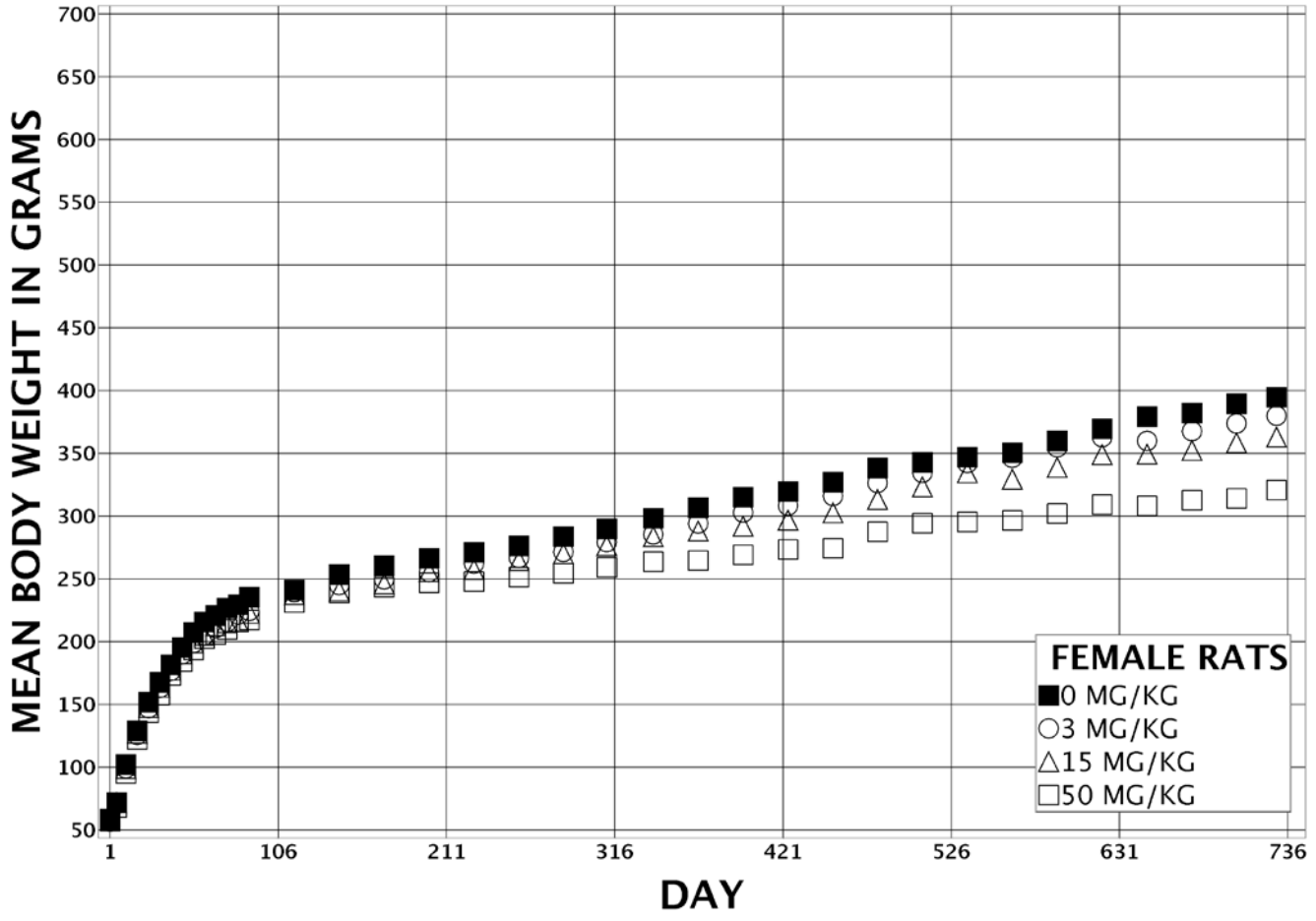
Dose (mg/kg)	0	3	15	50
Male Rat				
Survival	36	35	38	25*
Mean BW (g) % control (day 703)	673 -	669 (99%)	695 (103)	678 (101%)
Female Rat				
Survival	37	39	33	28
Mean BW (g) % control (day 704)	390 -	374 (96%)	358 (92%)	314 (81%)

*P≤0.05 N = 50 per group

Wistar Han Male Rat Body Weights



Wistar Han Female Rat Body Weights



DE-71 2-year study

Wistar Han Male Rat – Liver

Dose (mg/kg)	0	3	15	50
N	49	50	50	50
Hepatocellular adenoma[a]	3* (6%)	2 (4%)	4 (8%)	8 (16%)
Hepatocellular carcinoma[b]	0	0	0	2 (4%)
Hepatocellular adenoma or carcinoma[a]	3** (6%)	2 (4%)	4 (8%)	9* (18%)
Hepatocholangioma[b]	0*	0	0	2
Hepatocholangioma, hepatocellular adenoma or hepatocellular carcinoma[a]	3** (6%)	2 (4%)	4 (8%)	11* (22%)

*P≤0.05; **P≤0.01

Historical controls, gavage corn oil
[a]3/99 (3.1% ± 4.3%), range 0%-6%
[b]0/99

Historical controls, all routes
[a]4/299 (1.4% ± 2.5%), range 0%-6%
[b]0/299

Wistar Han Male Rat – Thyroid

Dose (mg/kg)	0	3	15	50
N	45	45	48	46
Thyroid gland follicle hypertrophy	1** (2%)	26** (58%)	34** (70%)	23** (50%)
Thyroid gland: follicular cell adenoma[a]	1* (2%)	3 (7%)	2 (4%)	6* (13%)
Thyroid gland: follicular cell carcinoma[b]	0 (0%)	2 (4%)	1 (2%)	0 (0%)
Thyroid gland follicular cell adenoma or carcinoma[a]	1 (2%)	5 (11%)	3 (6%)	6* (13%)

*P<0.05; **P<0.01

Historical controls, gavage corn oil
[a]4/95 (4.1% ± 2.7%), range 2%-6%
[b] 0/95 [a]

Historical controls, all routes
[a]5/295 (1.7% ± 2.4%), range 0%-6%
[b]0/295

Wistar Han Male Rat - Pituitary

Dose (mg/kg)	0	3	15	50
N	49	49	50	50
Pituitary gland: pars distalis or unspecified site adenoma[a]	19** (39%)	12 (24%)	22 (44%)	35** (70%)

**P<0.01

Historical controls, gavage corn oil
[a]40/99 (40.4% ± 2.3%),range 39%-42%

Historical controls, all routes
[a]101/298 (33.9% ± 5.7%), range 28%-42%

DE-71 2-year study

Wistar Han Female Rat - Liver

Dose (mg/kg)	0	3	15	50
N	50	49	50	47
Hepatocellular adenoma[a]	3** (6%)	2 (4%)	8 (16%)	16** (34%)
Hepatocellular carcinoma[b]	0**	0	1 (2%)	6** (13%)
Hepatocellular adenoma or carcinoma[a]	3** (6%)	2 (4%)	8 (16%)	17** (36%)
Cholangiocarcinoma[b]	0*	0	0	2 (4%)
Hepatocholangioma[b]	0**	0	0	8** (17%)
Hepatocholangioma, hepatocellular adenoma, or hepatocellular carcinoma[a]	3** (6%)	2 (4%)	8 (16%)	21** (45%)

Historical controls, gavage corn oil
[a]4/100 (4.0% ± 2.8%), range 2%-6%
[b]0/100

Historical controls, all routes
[a]6/300 (2.0% ± 2.2%), range 0%-6%
[b]0/300

*P≤0.05; **P≤0.01

Wistar Han Female Rat – Uterus

Original Transverse & Residual Longitudinal Review

Dose (mg/kg)	0	3	15	50
N	50	50	50	49
Uterus, metaplasia, squamous	0	2 (4%)	5* (10%)	6* (12%)
Cervix, squamous hyperplasia	2** (4%)	3 (6%)	4 (8%)	8* (16%)
Uterus polyp, stromal	4 (8%)	12* (24%)	11* (22%)	9 (18%)
Uterus, stromal sarcoma	0	0	1 (2%)	0
Uterus stromal polyp or stromal sarcoma[a]	4 (8%)	12* (24%)	12* (24%)	9 (18%)
Vaginal polyp	0*	0	0	2 (4%)

*P≤0.05; **P≤0.01

Historical controls, all routes

[a]29/194 (15.1% ± 6.3%), range 8%-22%

DE-71 2-year Study – Male Rats

Organ	Nonneoplastic Lesion
Liver	Eosinophilic focus
	Hepatocyte hypertrophy
	Fatty change
Thyroid gland	Follicle hypertrophy
Kidney	Hydronephrosis
Parotid salivary gland	Atrophy
	Cytoplasmic vacuolization
Prostate gland	Inflammation, chronic active
Preputial gland	Duct, ectasia
Thymus	Atrophy
Forestomach	Epithelium, hyperplasia

DE-71 2-year Study - Female Rats

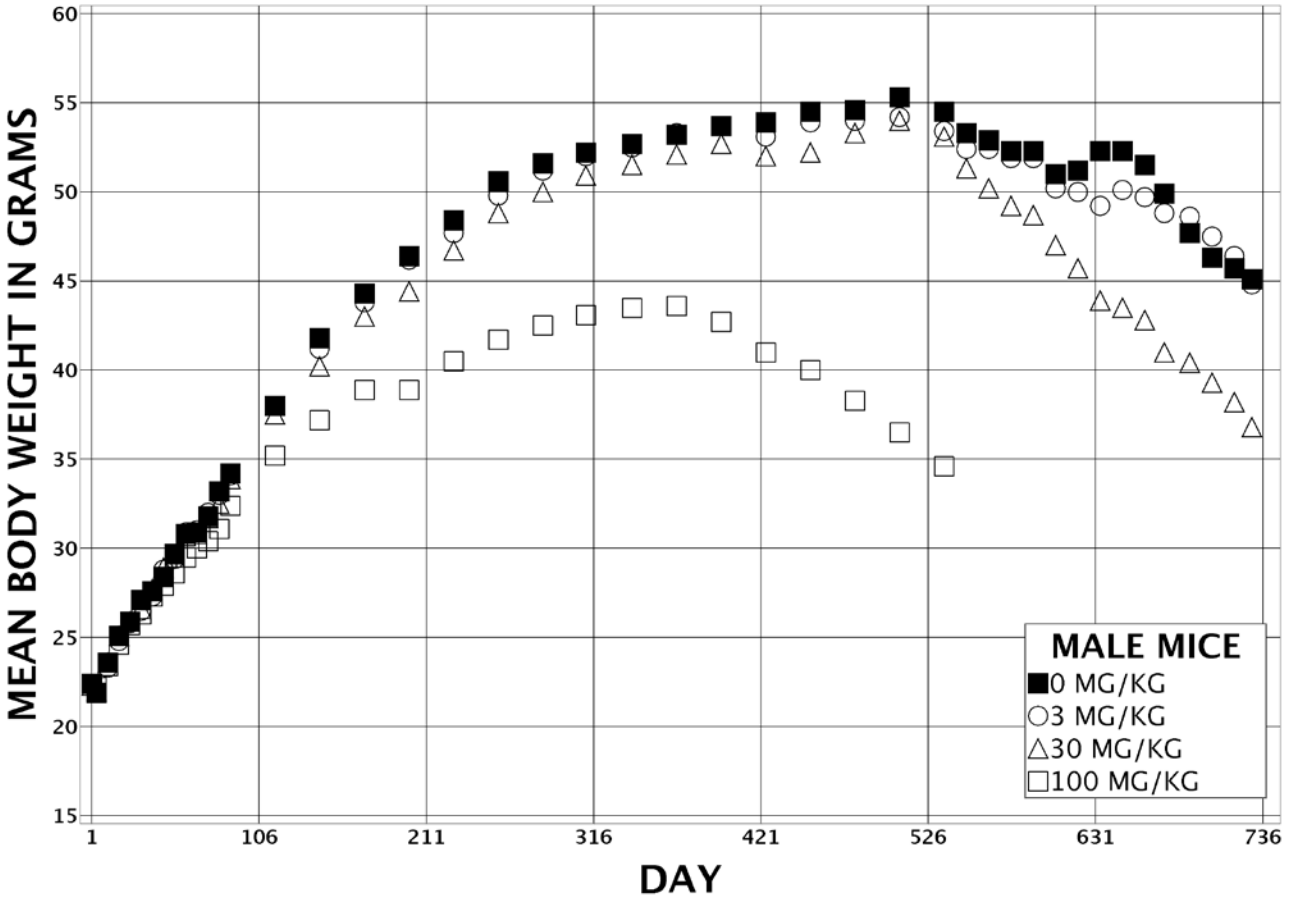
Organ	Nonneoplastic Lesion
Liver	Hyperplasia, nodular
	Eosinophilic focus
	Hepatocyte hypertrophy
	Fatty change
	Oval cell, hyperplasia
Thyroid gland	Follicle hypertrophy
	Follicular cell hyperplasia
Uterus	Squamous metaplasia
Cervix	Squamous hyperplasia
Kidney	Hydronephrosis
Adrenal cortex	Focal hyperplasia

B6C3F1/N Mice - Final Survival & Mean Body Weight

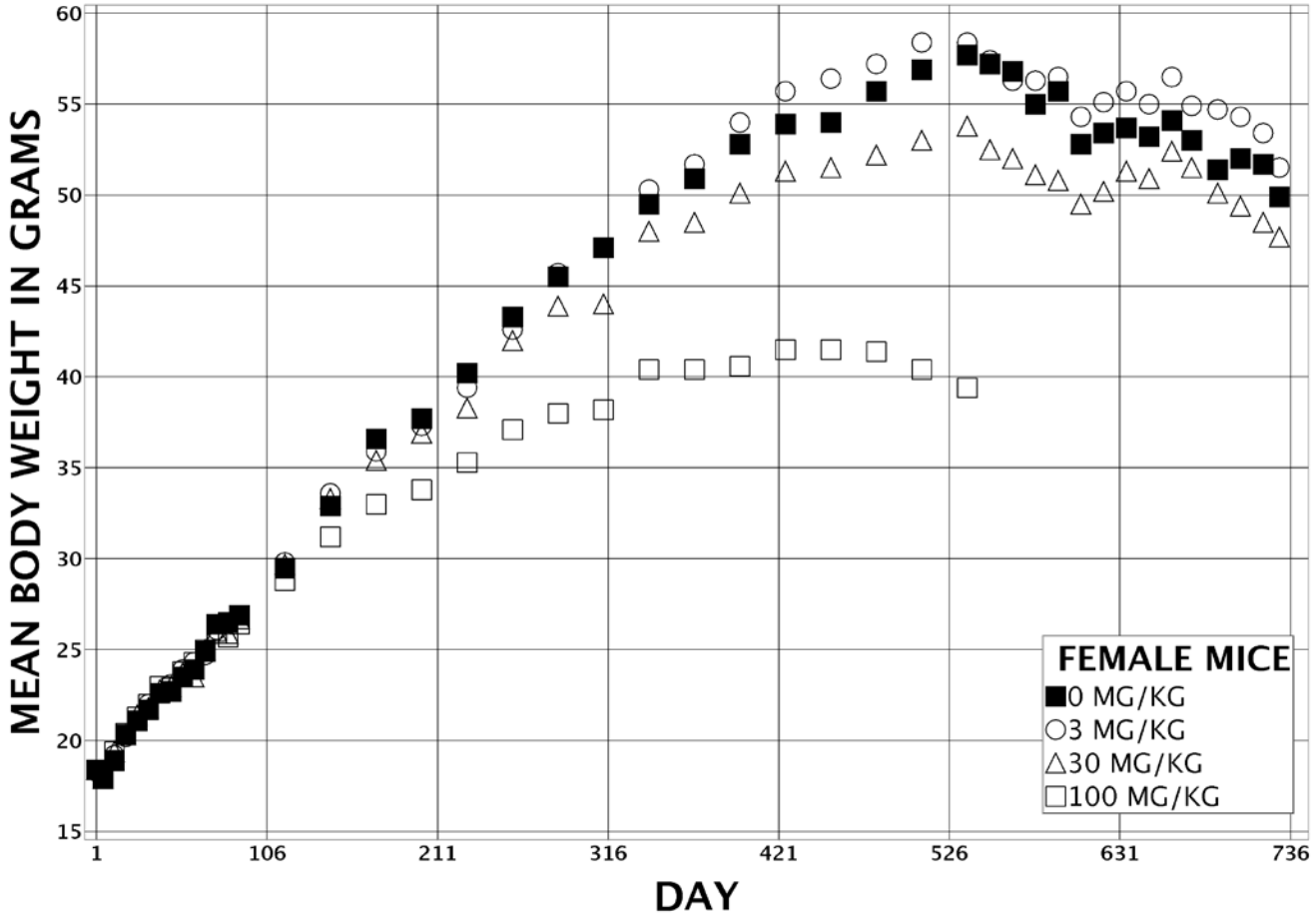
Dose (mg/kg)	0	3	30	100
Male Mice				
Survival	29	33	31	18 month termination
Mean BW (g) % control (day 718)	45.7 -	46.4 102%	38.2 84%	
Female Mice				
Survival	33	35	37	18 month termination
Mean BW (g) % control (day 719)	51.7 -	53.4 103%	48.5 94%	

N=50

B6C3F1/N Male Mouse Body Weights



B6C3F1/N Female Mouse Body Weights



B6C3F1/N Male Mouse - Liver

Dose (mg/kg)	0	3	30	100
N	50	50	50	50
Hepatocellular adenoma[a]	23** (46%)	35* (70%)	49** (98%)	40** (80%)
Hepatocellular carcinoma[b]	18** (36%)	15 (30%)	30* (60%)	45** (90%)
Hepatoblastoma[c]	1** (2%)	1 (2%)	16** (32%)	5* (10%)
Hepatocellular adenoma, carcinoma, or hepatoblastoma[d]	31** (62%)	40 (80%)	49** (98%)	47** (94%)

*P<0.05; **P<0.01

Historical controls, gavage corn oil
[a]168/300 (56.0% ± 6.7%),range 46%-64%
[b]105/300 (35.0% ± 9.8%), range 22%-44%
[c]10/300 (3.3% ± 2.4%), range 0%-6%
[d]221/300 (73.7% ± 6.1%), range 62%-78%

Historical controls, all routes
[a]437/700 (62.4% ± 10.5%), range 46%-78%
[b]262/700 (37.4% ± 11.2%), range 22%-52%
[c]34/700 (4.9% ± 3.7%), range 0%-12%
[d]545/700 (77.3% ± 8.3%), range 62%-90%

B6C3F1/N Female Mouse - Liver

Dose (mg/kg)	0	3	30	100
N	50	49	50	49
Hepatocellular adenoma[a]	5** (10%)	7 (14%)	32** (64%)	46** (94%)
Hepatocellular carcinoma[b]	4** (8%)	2 (4%)	6 (12%)	27** (55%)
Hepatocellular adenoma or carcinoma[c]	8** (16%)	8 (16%)	33** (66%)	47** (96%)

**P<0.01

Historical controls, gavage corn oil
[a]67/300 (22.3% ± 10.5%), range 10%-34
[b]30/300 (10.0% ± 5.1%), range 4%-18%
[c]85/300 (28.3% ± 10.2%), range 16%-40%

Historical controls, all routes
[a]272/698 (39.1% ± 21.9%), range 62%-90%
[b]112/698 (16.1% ± 8.1%), range 4%-34%
[c]320/698 (45.9% ± 21.9%), range 16%-82%

DE-71 2-year Study – Male Mouse

Organ	Nonneoplastic Lesion
Liver	Centrilobular, hypertrophy
	Clear cell focus
	Necrosis, focal
	Kupffer cell pigmentation
Thyroid gland	Follicle hypertrophy
Forestomach	Epithelium, hyperplasia
	Inflammation
Adrenal cortex	Hypertrophy, diffuse
Testes	Germinal epithelium, atrophy

DE-71 2-year Study – Female Mouse

Organ	Nonneoplastic Lesion
Liver	Centrilobular, hypertrophy
	Eosinophilic focus
	Fatty change
	Kupffer cell pigmentation
Thyroid gland	Follicle hypertrophy
Forestomach	Epithelium, hyperplasia
Adrenal cortex	Hypertrophy, diffuse

DE-71 2-year Conclusions

- **Male Wistar Han rat [Crl:WI(Han)]**
  - **Clear** evidence of carcinogenic activity – hepatocholangioma, hepatocellular adenoma, or hepatocellular carcinoma (combined)
  - Thyroid gland follicular cell adenoma or carcinoma and pituitary gland (pars distalis) adenoma were also considered related to exposure
- **Female Wistar Han rat [Crl:WI(Han)]**
  - **Clea**r evidence of carcinogenic activity – hepatocholangioma, hepatocellular adenoma, and hepatocellular carcinoma
  - Cholangiocarcinoma was also considered related to exposure
  - Uterine stromal polyp or stromal sarcoma (combined) may have been related to exposure

DE-71 2-year Study Conclusions

- **Male B6C3F1/N mice**
  - **Clear** evidence of carcinogenic activity – hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma
- **Female B6C3F1/N mice**
  - **Clea**r evidence of carcinogenic activity – hepatocellular adenoma and hepatocellular carcinoma

DE-71 2-year Study Conclusions

Treatment-related Nonneoplastic Lesions

- **Male rats** – liver, thyroid gland, kidney, parotid salivary gland, prostate gland, preputial gland, thymus, forestomach
- **Female rats** – liver, thyroid gland, uterus, cervix, kidney, adrenal cortex
- **Male mice** –liver, thyroid gland, forestomach, adrenal cortex, testes
- **Female mice** – liver, thyroid gland, forestomach, adrenal cortex

Other study findings

- Incidence of female rat liver tumors was not related to AhR receptor polymorphism
- Liver tumor mutation analysis
  - Rat hepatocellular tumors from DE-71 exposure harbored a unique *Hras* mutation at codon 60 but not at the usual hotspot codon 61
  - Mouse hepatocellular carcinomas from DE-71 exposure had a significant increase in *Ctnnb1* incidence
- BDE tissue level increased with increasing dose