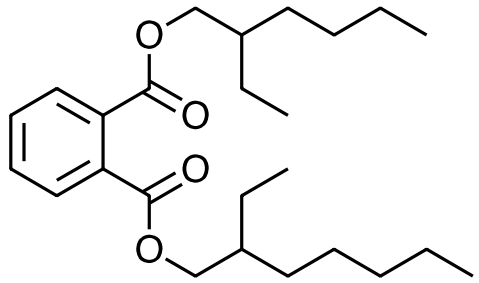
NTP
National Toxicology Program

NTP Technical Report on the Toxicology and Carcinogenesis Studies of Di(2-ethylhexyl) Phthalate (CASRN 117-81-7) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats



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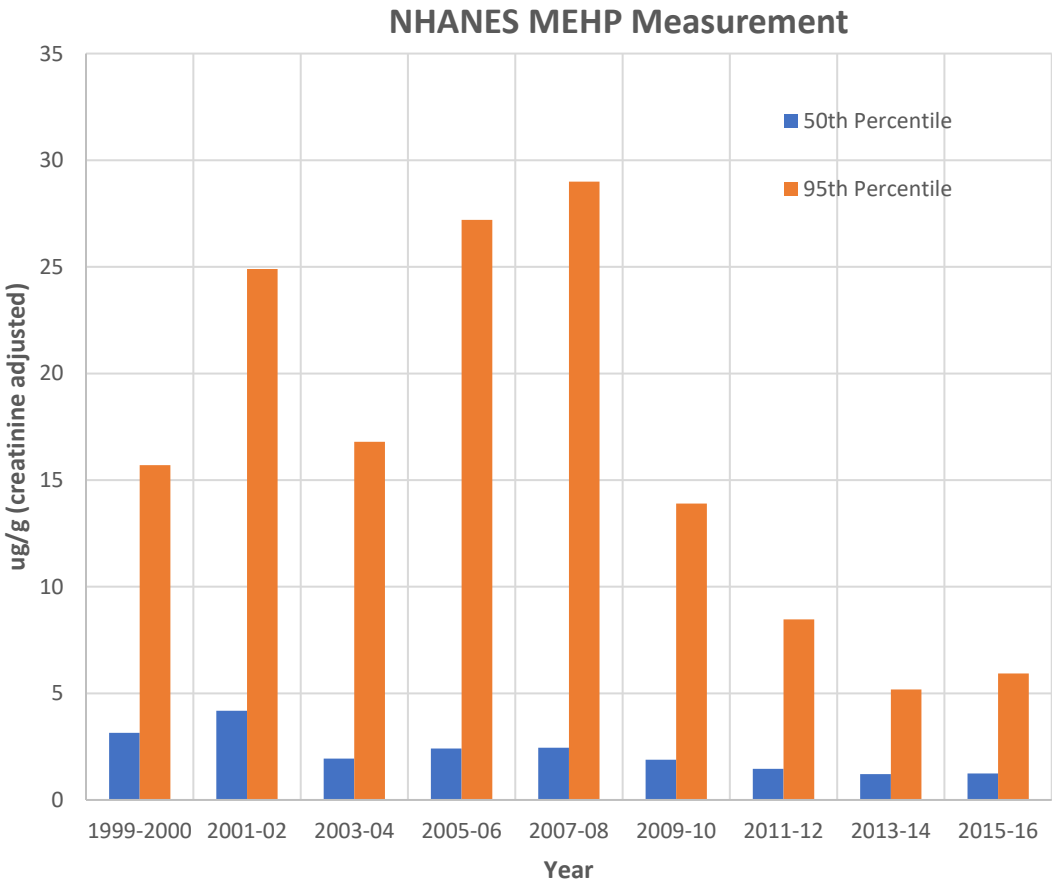
Study Background

- Di(2-ethylhexyl) phthalate (DEHP) is a phthalate ester that had widespread use in manufacturing of PVC polymers and corresponding products (e.g. cosmetics, toys).
- While use of DEHP has declined due to toxicity concerns, there was chronic exposure to DEHP throughout multiple life stages.
- Literature suggests that early life exposure to DEHP may affect chronic or carcinogenic outcomes.



Study Rationale

- In utero exposure to DEHP metabolites occurs in humans.
- Sensitivity to adverse developmental effects to the male rat reproductive system.
- Previous DEHP chronic rodent studies did not include exposure during the gestational period up to weaning in rodents (perinatal).
- NTP conducted comparative DEHP carcinogenic studies to determine if exposure paradigm, i.e. including early life exposure, alters chronic toxicity or carcinogenicity outcome.

Design

- Two carcinogenicity studies conducted in rats to compare and contrast:
  - Study 1: exposure started during gestation – “Perinatal + post-weaning exposure”
  - Study 2: exposure started at weaning – “Post-weaning exposure”

- In these studies, DEHP was administered in dosed feed to mimic a common route of human exposure.

- Necropsy included an evaluation of the reproductive tract in males with the perinatal exposure. In addition, gestational exposure was quantified.

Exposure Selection

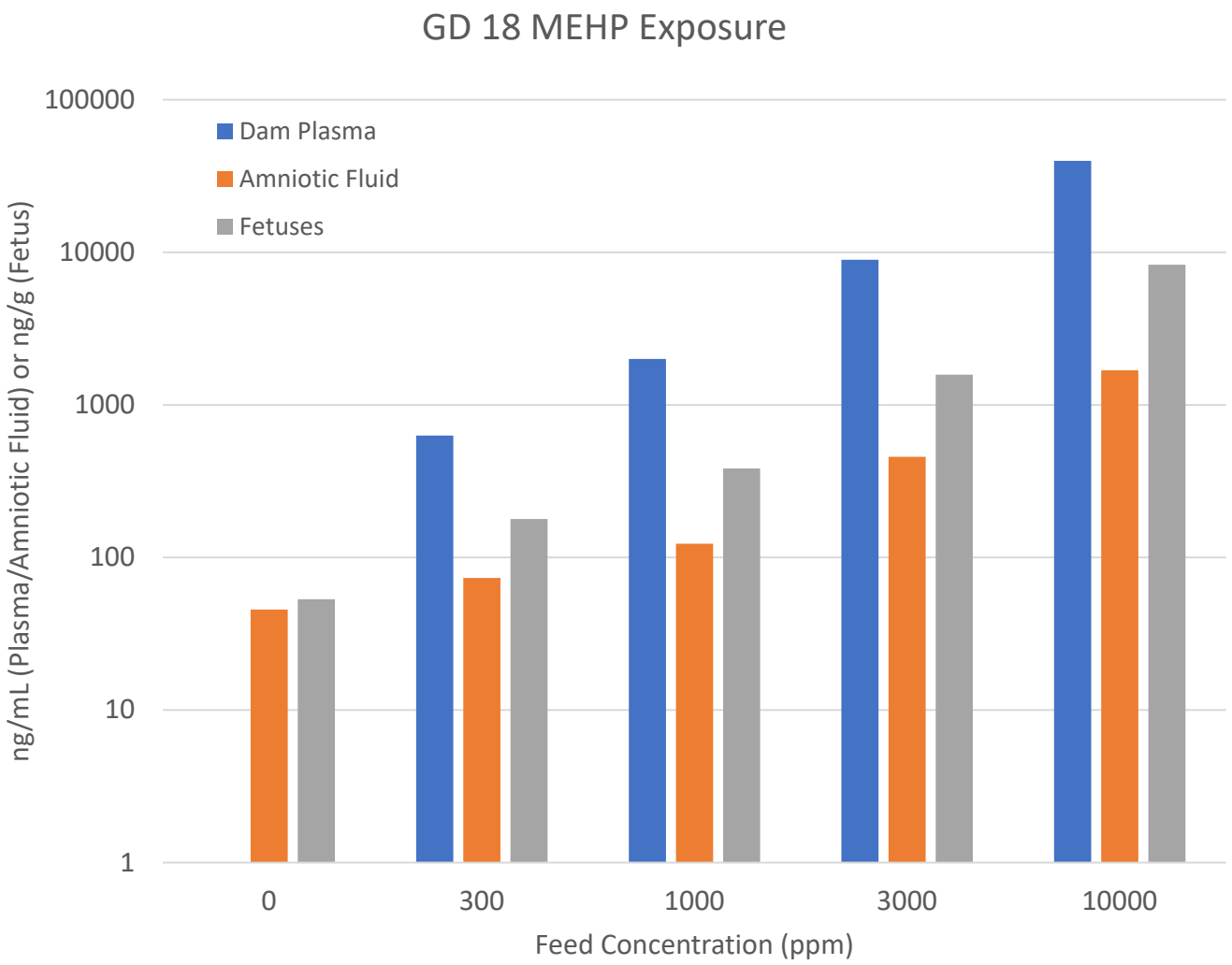
- Feed concentrations based on previous studies:
  - Reproductive assessment by continuous breeding (RACB): 10000 ppm in Sprague Dawley (SD) rats induced reproductive tract malformations, but no effect on litter size or survival.
  - NTP TR-217: Carcinogenicity study of 6000 and 12000 ppm in F344/N rats resulted in hepatocellular neoplasms.
- Feed concentrations selected were: 0, 300, 1000, 3000, or 10000 ppm.

Chemical consumption (mg/kg/day)

	300 ppm	1000 ppm	3000 ppm	10000 ppm
Perinatal and Postweaning				
Gestation (F0)	21	68	206	626
Lactation (F0; LD 1-14)	49	166	482	1244
Postweaning (F1 Males)	18	58	189	678
Postweaning (F1 Females)	18	62	196	772
Postweaning				
Gestation (F0)	-	-	-	-
Lactation (F0; LD 14-21)	-	-	-	-
Postweaning (F1 Males)	17	54	170	602
Postweaning (F1 Females)	17	60	177	646

LD = Lactation Day

Perinatal Exposure (MEHP)



- Moderate gestational transfer
- Potential saturation of MEHP clearance at higher concentrations
  - 3.0 fold increase in DEHP consumption vs 4.4 fold increase in dam plasma MEHP concentration

Results: Perinatal Exposure (GD 6 – PND 21)

- Dam weights were decreased during gestation and lactation in the 10000 ppm group (up to 10% and 25% respectively compared to controls).

- Decreased litter size in 10000 ppm group at PND 1 compared to controls (12.6 vs 10.5 pups/litter). Survival unaffected except in 3000 ppm group PND 5 to 21 compared to controls (96.2% vs 99.0% respectively).

- F1 pup weights were significantly reduced in the 10000 ppm group (up to 55%) during lactation, while differences in groups ≤ 3,000 ppm were ≤ 6% compared to controls.

PND = postnatal day

Postweaning Period

Exposure	Sex	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
Survival (percent)						
Perinatal + Postweaning	Male	50.0	67.3	80.0**	70.0	58.0
	Female	62.0	64.0	68.0	68.0	54.0
Postweaning	Male	64.0	68.0	78.0	70.0	84.0*
	Female	66.0	68.0	66.0	68.0	64.0
Weights (percent of control)						
Perinatal + Postweaning	Male	-	100.8	102.3	98.0	70.3
	Female	-	99.1	104.6	90.1	68.3
Postweaning	Male	-	103.7	102.9	99.0	84.4
	Female	-	104.3	105.9	96.9	78.1

* $p < 0.05$; ** $p < 0.01$

Nonneoplastic Lesions

Lesion	Male (P+P)	Male (P)	Female (P+P)	Female (P)
Hepatocyte Cytoplasmic Alteration	↑	↑	↑	↑
Hepatocyte Hypertrophy	↑	↑	↑	↑
Pigment	↑	↑	↑	↑
Necrosis	↑	↑	-	-
Eosinophilic Focus	↑	↑	↑	-
Basophilic Focus	↑	-	↑	-
Clear Cell Focus	-	↑	-	-
Bile Duct Hyperplasia	-	-	↑	-

P+P = Perinatal and Postweaning Exposure
P = Postweaning Exposure

Liver Response (Males)

Neoplasms

Exposure	Lesion	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
Perinatal+Postweaning	Hepatocellular Adenoma	0/50**	1/49	0/50	3/50	8/49*
	Hepatocellular Carcinoma	1/50	0/49	0/50	0/50	3/49
	Hepatocellular Adenoma or Carcinoma	1/50	1/49	0/50	3/50	11/49**
Postweaning	Hepatocellular Adenoma	0/50**	2/50	0/50	1/50	6/50*
	Hepatocellular Carcinoma	0/50**	0/50	0/50	0/50	6/50*
	Hepatocellular Adenoma or Carcinoma	0/50**	2/50	0/50	1/50	12/50**

* $p < 0.05$; ** $p < 0.01$ (Rao-Scott/Poly-3)
Historical Control
Adenoma: 2/489, 0/50 - 1/50
Carcinoma: 2/489, 0/50 - 1/50
Adenoma or Carcinoma: 4/489, 0/50 - 1/50

Clear Evidence of Carcinogenic Activity in both exposure paradigms

Liver Response (Females)

Neoplasms

Exposure	Lesion	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
Perinatal+Postweaning	Hepatocellular Adenoma	1/49	0/50	5/50	9/50*	5/48
	Hepatocellular Carcinoma	0/49**	0/50	0/50	0/50	8/48**
	Hepatocellular Adenoma or Carcinoma	1/49**	0/50	5/50	9/50*	13/48**
Postweaning	Hepatocellular Adenoma	0/50**	0/50	1/50	1/50	13/49**
	Hepatocellular Carcinoma	0/50	0/50	0/50	0/50	2/49
	Hepatocellular Adenoma or Carcinoma	0/50**	0/50	1/50	1/50	14/49**

* $p < 0.05$; ** $p < 0.01$ (Rao-Scott/Poly-3)

Historical Control

Adenoma: 15/489, 0/50 – 4/50

Carcinoma: 1/489, 0/50 – 1/50

Adenoma or Carcinoma: 16/489, 0/50 – 4/50

Clear Evidence of Carcinogenic Activity in both exposure paradigms

Pancreas Response (Males)

Exposure	Lesion	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
Perinatal+Postweaning	Acinus Hyperplasia	13/50	9/49	16/50	25/50	15/49
	Acinar Adenoma	10/50**	7/49	8/50	36/50**	22/49**
	Acinar Carcinoma	0/50	0/49	0/50	3/50	1/49
	Acinar Adenoma or Carcinoma	10/50**	7/49	8/50	38/50**	22/49**
Postweaning	Acinus Hyperplasia	7/49**	8/50	9/50	24/50**	26/50**
	Acinar Adenoma	1/49**	4/50	5/50	23/50**	30/50**
	Acinar Carcinoma	0/49**	1/50	0/50	1/50	5/50*
	Acinar Adenoma or Carcinoma	1/49**	5/50	5/50	23/50**	33/50**

* $p < 0.05$; ** $p < 0.01$ (Rao-Scott/Poly-3)
Historical Control
Adenoma: 60/488, 0/50 – 14/50
Carcinoma: 4/488, 0/50 – 2/50
Adenoma or Carcinoma: 62/488, 0/50 – 14/50

Clear Evidence of Carcinogenic Activity in both exposure paradigms

Pancreas Response (Females)

Exposure	Lesion	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
Perinatal+Postweaning	Acinus Hyperplasia	0/49	0/50	0/50	2/50	3/48
	Acinar Adenoma	0/49	0/50	0/50	2/50	1/48
	Acinar Carcinoma	0/49	0/50	0/50	0/50	0/48
	Acinar Adenoma or Carcinoma	0/49	0/50	0/50	2/50	1/48
Postweaning	Acinus Hyperplasia	0/50**	1/50	1/50	1/50	5/47*
	Acinar Adenoma	0/50	0/50	0/50	1/50	1/47
	Acinar Carcinoma	0/50	0/50	0/50	0/50	1/47
	Acinar Adenoma or Carcinoma	0/50*	0/50	0/50	1/50	2/47

* $p < 0.05$; ** $p < 0.01$ (Rao-Scott/Poly-3)
Historical Control
Adenoma: 0/489
Carcinoma: 0/489
Adenoma or Carcinoma: 0/489

Related to Exposure (Some Evidence) in both exposure paradigms

Reproductive Tract Malformations

- Perinatal + postweaning exposure study resulted in malformations primarily in the 10000 ppm group.
- Males:
  - Testis: Small, undescended, not present
  - Epididymis: Small, agenesis (caput/corpus/cauda), not present
  - LABC, Cowper's Glands, Prostate, Seminal Vesicles/Coagulating Glands: Small
  - Prepuce: Cleft, incomplete preputial separation
  - Gubernaculum: Extended
- Females:
  - Vagina: Not patent
  - Phallus: Cleft

Male Reproductive Response

Exposure	Lesion	P+P	P
Testis	Germinal epithelium, degeneration	↑	↑
	Interstitial cell, hyperplasia, focal	↑	↑
	Seminiferous tubule, dysgenesis	↑	-
	Edema	-	↑
Epididymis	Hypospermia	↑	↑
	Duct, exfoliated germ cell	-	↑

P+P = Perinatal and Postweaning Exposure
P = Postweaning Exposure

Testis Response

Exposure	Lesion	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
Perinatal+Postweaning	Interstitial Cell Hyperplasia	4/49**	3/49	6/50	5/50	30/49**
	Interstitial Cell Adenoma	3/49	1/49	3/50	5/50	5/49
Postweaning	Interstitial Cell Hyperplasia	1/50*	1/50	0/50	4/50	4/50
	Interstitial Cell Adenoma	7/50**	3/50	3/50	6/50	15/50

* $p < 0.05$; ** $p < 0.01$ (Rao-Scott/Poly-3)
HC Adenoma: 19/487, 0/50 – 7/50

No carcinogenic effect with perinatal + postweaning exposure

May be related to exposure (Equivocal Evidence) with postweaning-only exposure

Uterine Response

Exposure	Lesion	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
Perinatal+Postweaning	Adenoma	0/50	1/50	0/50	0/50	0/50
	Adenocarcinoma	3/50**	0/50	1/50	3/50	6/50
	Squamous Cell Carcinoma	0/50	1/50	0/50	0/50	1/50
	Squamous Cell Papilloma	0/50	0/50	0/50	1/50	0/50
	Combination	3/50**	1/50	1/50	3/50	7/50
Postweaning	Adenoma	0/50	1/50	0/50	0/50	0/49
	Adenocarcinoma	2/50**	2/50	1/50	4/50	10/50*
	Squamous Cell Carcinoma	0/50	1/50	0/50	2/50	1/50
	Squamous Cell Papilloma	0/50	0/50	0/50	0/50	2/50
	Combination	2/50**	4/50	1/50	6/50	13/50**

* $p < 0.05$; ** $p < 0.01$ (Rao-Scott/Poly-3)
Historical Control
Adenocarcinoma: 20/350, 1/50 – 5/50
Combination: 23/350, 1/50 – 5/50

May be related (equivocal evidence) with perinatal+postweaning exposure

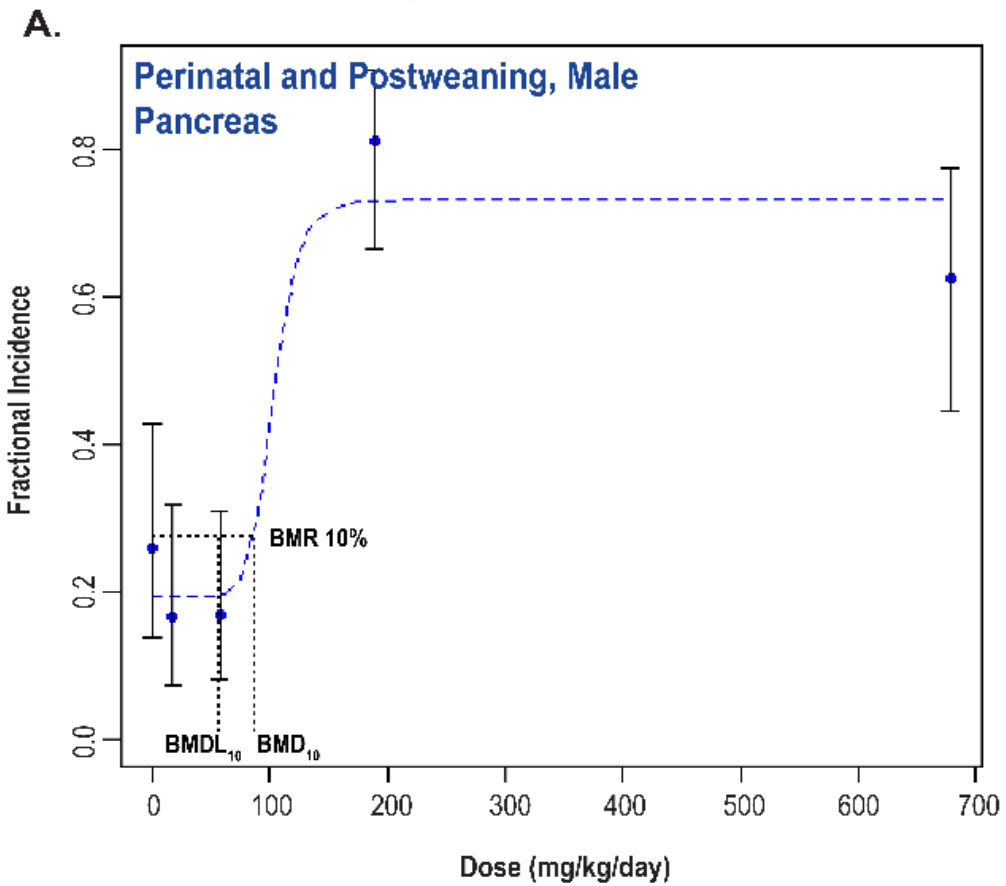
Clear evidence of carcinogenic activity with postweaning only exposure

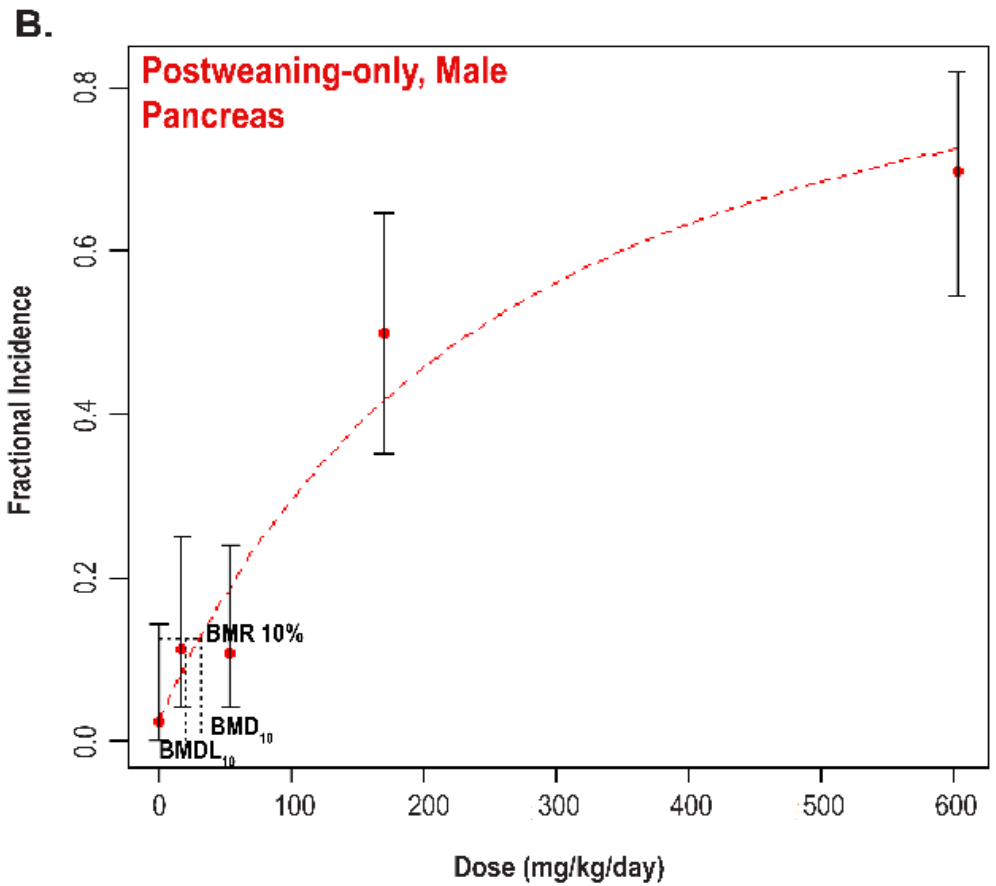
Other Nonneoplastic Lesions

Organ	Lesion	Male (P+P)	Male (P)	Female (P+P)	Female (P)
Heart	Valve Fibrosis	↑	↑	-	-
	Valve thrombus	↑	↑	-	-
Bone Marrow	Hypercellularity	↑	↑	-	↑
Pituitary Gland	Pars Distalis Hypertrophy	↑	↑	-	-
Kidney	Papilla Edema	↑	-	↑	-
	Papilla Hemorrhage	↑	-	↑	-
	Papilla Epithelium Hyperplasia	↑	-	↑	-
	Infarct	↑	-	↑	-
	Renal Tubule Cyst	-	-	↑	-
	Renal Tubule Dilation	-	-	↑	-

P+P = Perinatal and Postweaning Exposure
P = Postweaning Exposure

Comparative Carcinogenic Benchmark Dose Analysis





Carcinogenic Response BMD_{10} (mg/kg/day) Summary

Organ	Male (P+P)	Male (P)	Female (P+P)	Female (P)
Liver	383	434	123	384
Pancreas	86	31	-	-
Testis	-	367	-	-
Uterus	-	-	594	324

Lowest BMD_{10} was pancreas response in males with postweaning exposure

BMD_{10}'s within 1-3 fold of each other between exposure paradigms

No consistent pattern indicating one exposure was more sensitive than the other

Discussion

- Carcinogenic findings generally consistent with NTP TR-217, David et al. 2000, Voss et al. 2005, except the uterine findings are new.
- Other studies have identified PPARα and non-PPARα mechanisms involved with hepatocellular carcinogenic activity of DEHP.
- Although reproductive malformations were observed, carcinogenic activity within reproductive organs not specific to perinatal and postweaning exposure.
- Findings are consistent with recent PFOA perinatal and postweaning comparison, which displayed marginal difference in carcinogenic response.

DEHP Conclusions

Perinatal and Postweaning Exposure

- Male
  - *Clear evidence of carcinogenic activity*
    - Increased incidences of hepatocellular adenoma or carcinoma (combined).
    - Increased incidences of acinar adenoma or carcinoma (combined) neoplasms (predominantly adenomas) of the pancreas.
  - Exposure to di(2-ethylhexyl) phthalate resulted in increased incidences of nonneoplastic lesions in the liver, heart, pituitary gland, testis, and epididymis and increased incidences of gross lesions of the reproductive tract, bone marrow, and kidney in male rats.
- Female
  - *Clear evidence of carcinogenic activity*
    - Increased incidence of hepatocellular adenoma or carcinoma (combined).
  - The occurrence of pancreatic acinar adenoma or carcinoma (combined) was considered to be related to exposure. *(Some Evidence)*
  - The occurrence of uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats may have been related to exposure. *(Equivocal Evidence)*
  - Exposure to di(2-ethylhexyl) phthalate resulted in increased incidences of nonneoplastic lesions in the liver and increased incidences of gross lesions of the kidney in female rats.

DEHP Conclusions

Postweaning Exposure

- Male
  - *Clear evidence of carcinogenic activity*
    - Increased incidences of hepatocellular adenoma or carcinoma (combined).
    - Increased incidences of acinar adenoma or carcinoma (combined) neoplasms (predominantly adenomas) of the pancreas.
  - The occurrence of testicular interstitial cell adenoma in male rats may have been related to exposure. (*Equivocal evidence*)
  - Exposure to di(2-ethylhexyl) phthalate resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, bone marrow, heart, pituitary gland, testis, and epididymis.
- Female
  - *Clear evidence of carcinogenic activity*
    - Increased incidences of hepatocellular adenoma or carcinoma (combined) and uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined).
  - The occurrence of pancreatic acinar adenoma or carcinoma (combined) in female rats was considered to be related to exposure. *(Some evidence)*
  - Exposure to di(2-ethylhexyl) phthalate resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, bone marrow, and uterus in female rats.

DEHP Conclusions

- No consistent pattern indicating that perinatal and postweaning exposure was more sensitive compared to postweaning-only exposure and modeled responses were within threefold of each other.

- However, there was a stronger carcinogenic response in the reproductive organs (uterus and testis) in the postweaning-only exposure study compared to the perinatal and postweaning exposure study.

Questions?