NTP
National Toxicology Program

Draft NTP Developmental and Reproductive Toxicity Technical Report on the Modified One-Generation Study of 2-Hydroxy-4-methoxybenzophenone (2H4MBP) (CASRN 131-57-7) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats with Prenatal and Reproductive Performance Assessments in F_1 Offspring

DART Report 05

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NTP's Interest in 2-Hydroxy-4-methoxybenzophenone (2H4MBP) and 2-Ethylhexyl *p*-methoxycinnamate (EHMC)

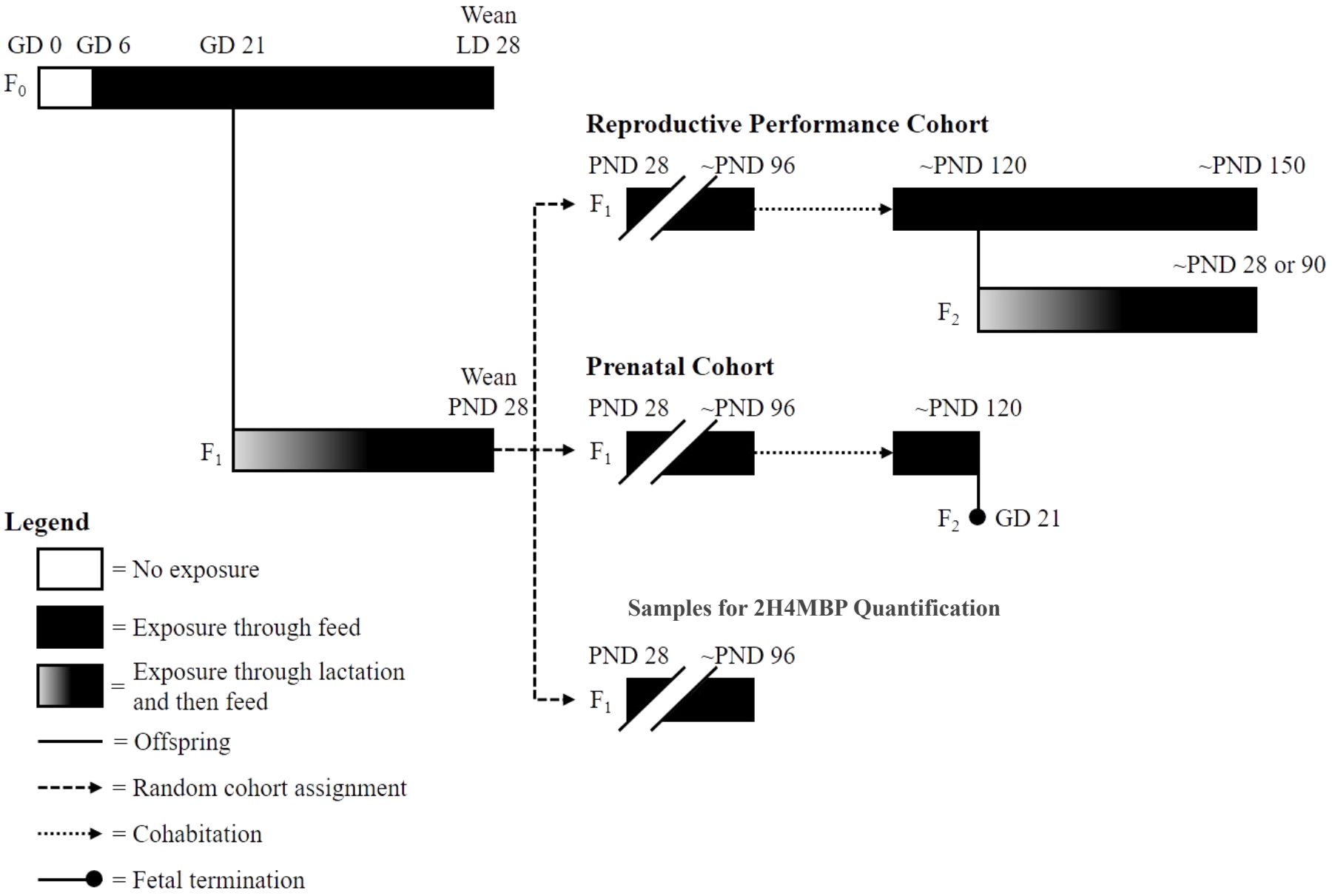
- Common UV filters in sunscreens
  - 2H4MBP is formulated up to 6%, EHMC up to 7.5%
  - Human 2H4MBP urinary levels (creatinine adjusted; 95th percentile) range from 350 to 2470 μg/g; with some children exhibiting values up 29100 μg/g
  - Reports of 'endocrine' signals

Characterization of Potential Toxicities to Address Knowledge Gaps

- Potential for adverse responses resulting from perinatal exposure
  - Hormonal signals have been reported; sufficient in magnitude to result in adverse outcomes?
    - *DOI: https://doi.org/10.22427/NTP-TR-597*
  - Potential for adverse reproductive and/or developmental outcomes (e.g., survival, growth, terata)?

- Bridging oral to dermal exposures
  - Pragmatically not possible to conduct littering dermal studies
  - Prefer to group house animals
  - Impact of vehicle selection on absorption

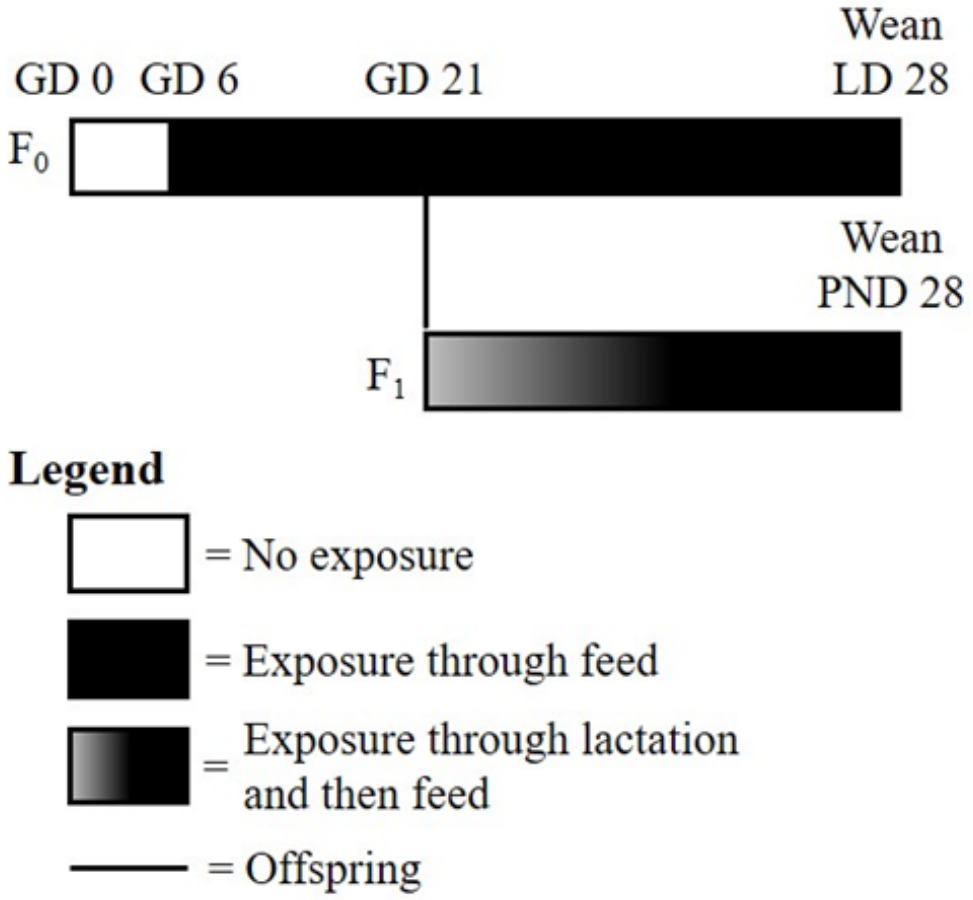
2H4MBP MOG Study Design



2H4MBP Dose Range-Finding Study Design

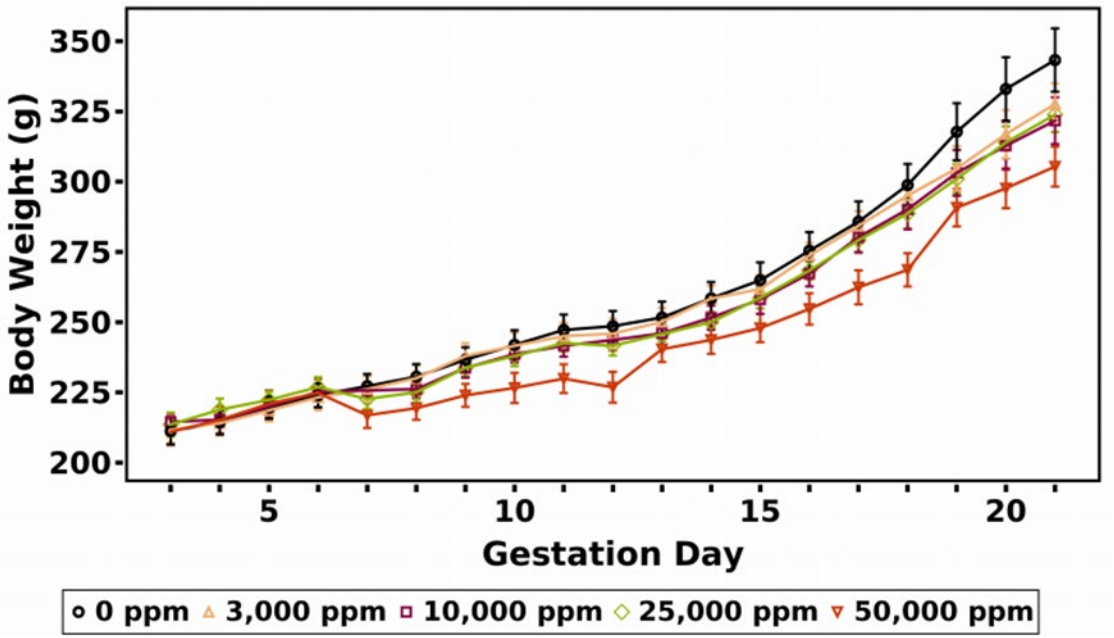
N = 8-9 time-mated dams

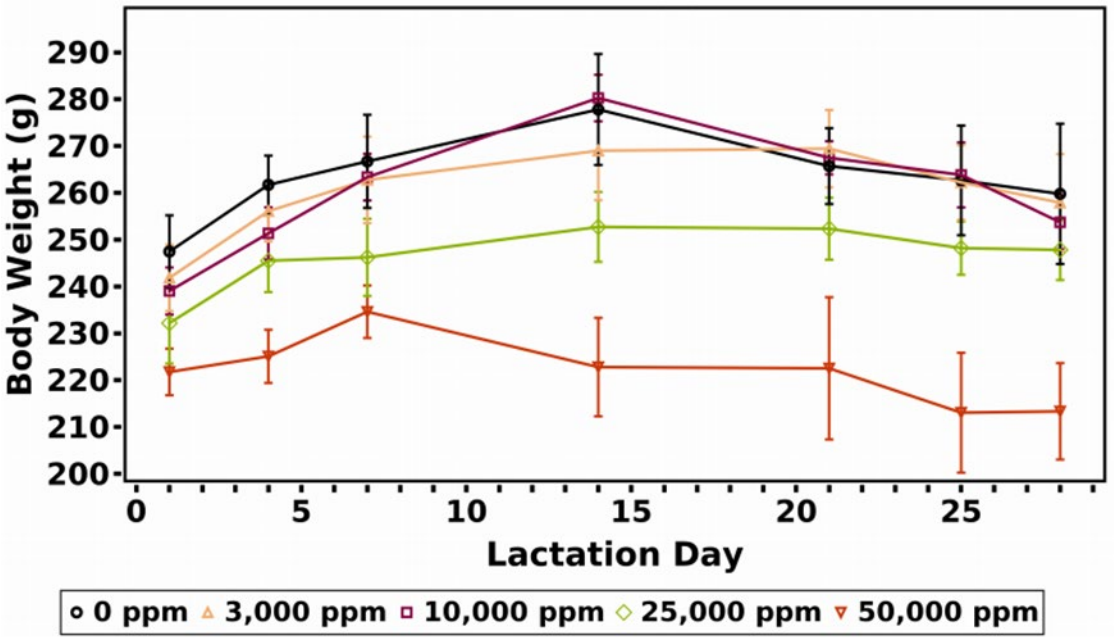
Extra dams were added for bioanalytical assay development



2H4MBP Dose Range-Finding Study Results

F_0 Dam Body Weights



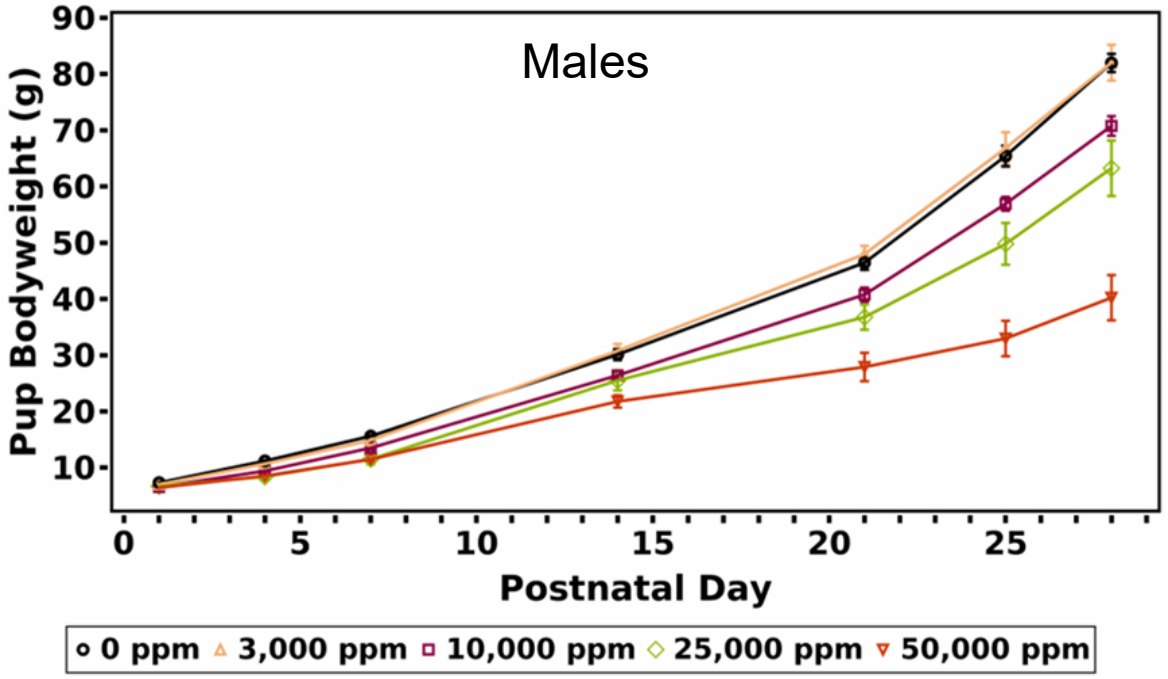


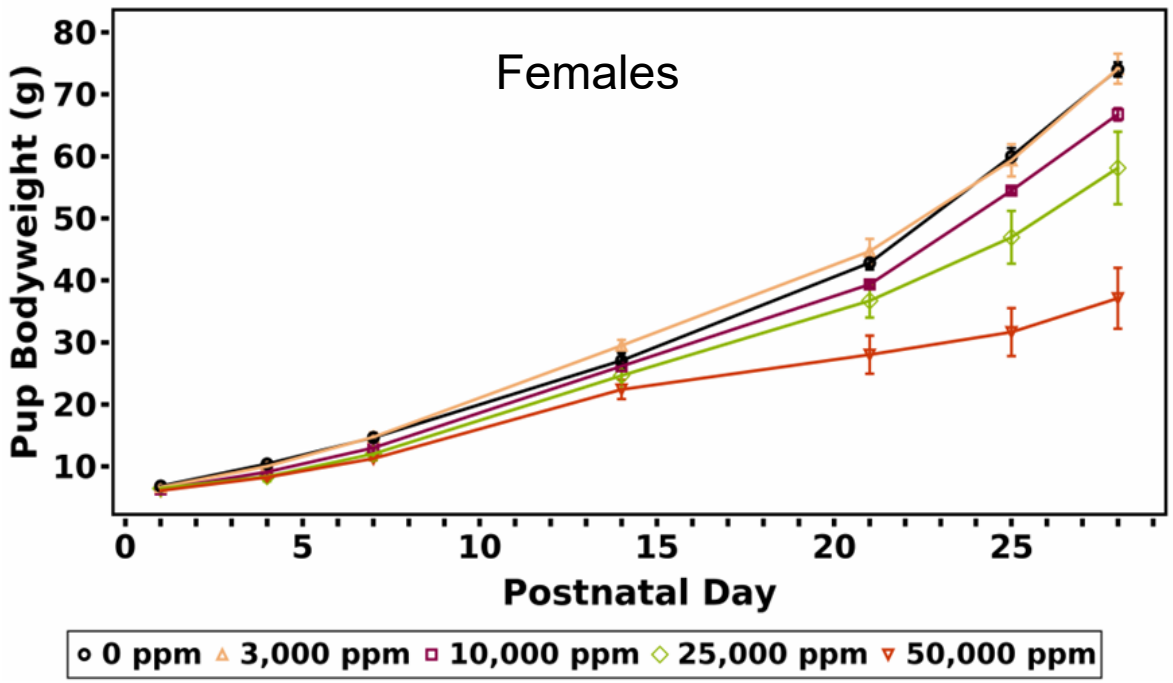
- Lower (~20%) relative body weights in the 50000 ppm exposure group
- Less of a response in the 10000 and 25000 ppm exposure groups

	Chemical Intake (mg/kg/day)				
	0 ppm	**3000 ppm**	**10000 ppm**	**25000 ppm**	**50000 ppm**
GD 6-21	0.0 ± 0.0	214.5 ± 4.7	695.2 ± 30.4	2,085.7 ± 161.2	6,426.4 ± 355.5
LD 1-14	0.0 ± 0.0	576.7 ± 18.4	1,858.3 ± 173.8	4,460.1 ± 310.8	12,028.5 ± 715.5

2H4MBP Dose Range-Finding Study Results

F_1 Pup Body Weights and Viability



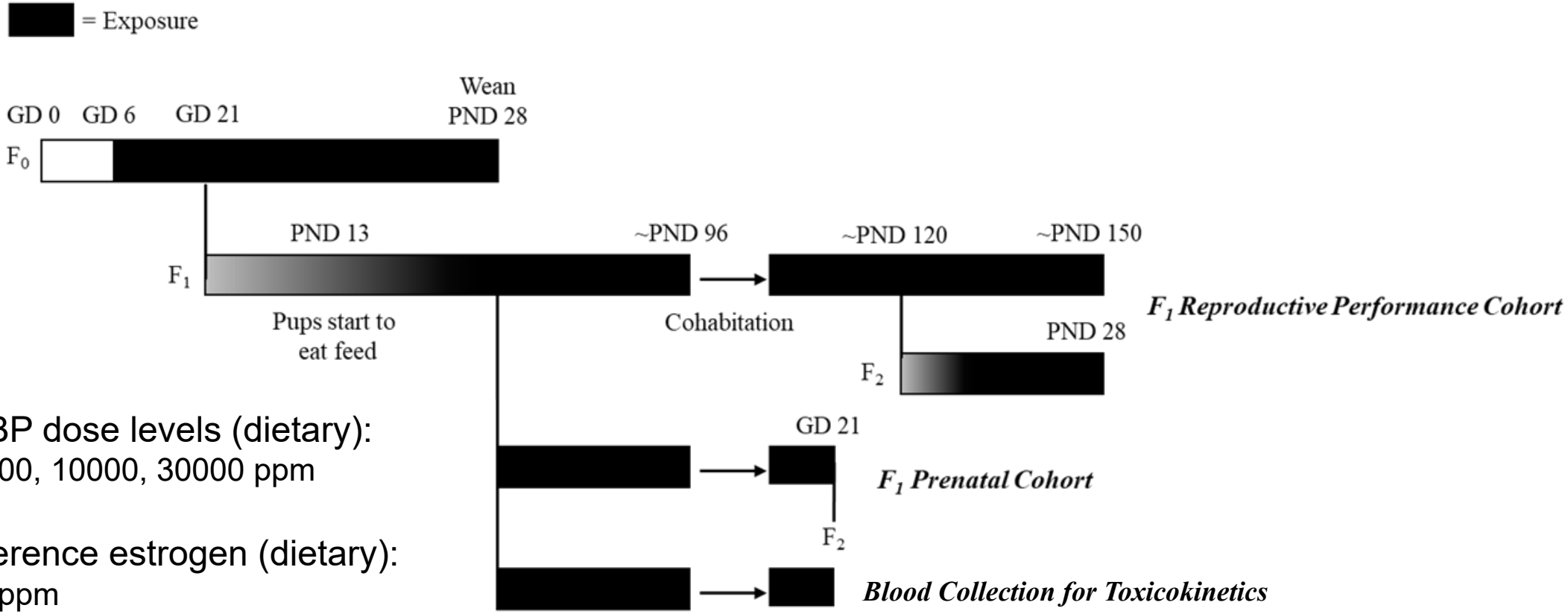


- No effects on gestational length or live litter size
- In the 50000 ppm group, PND 1 pup weights ~10% lower; lower relative pup weights, ↓ pup viability
- Pup body weights were lower in the 25000 ppm exposure group

Exposure Concentration Selection Rationale

- The selection of 30000 ppm 2H4MBP as the high exposure concentration was based on:
  - Toxicity observed at 50000 ppm
  - Apparent effect on body weight and marginal effect on pup survival at 25000 ppm (most of the pup deaths at this exposure concentration were attributed to a single dam)
- Exposure concentration and spacing (3000, 10000, 30000 ppm) were identified to achieve a no-observed-adverse-effect level and to avoid excessive overlap of the ingested doses due to increased feed consumption during pregnancy and lactation
- The selection of the 0.05 ppm ethinyl estradiol (EE) exposure concentration as a reference positive control was informed by the National Center for Toxicological Research studies, which demonstrated that this exposure concentration accelerated time to vaginal opening, delayed time to balanopreputial separation, caused transient alterations in estrous cyclicity, and induced male mammary gland hyperplasia
  - *Ethinyl Estradiol, NTP TR 547*

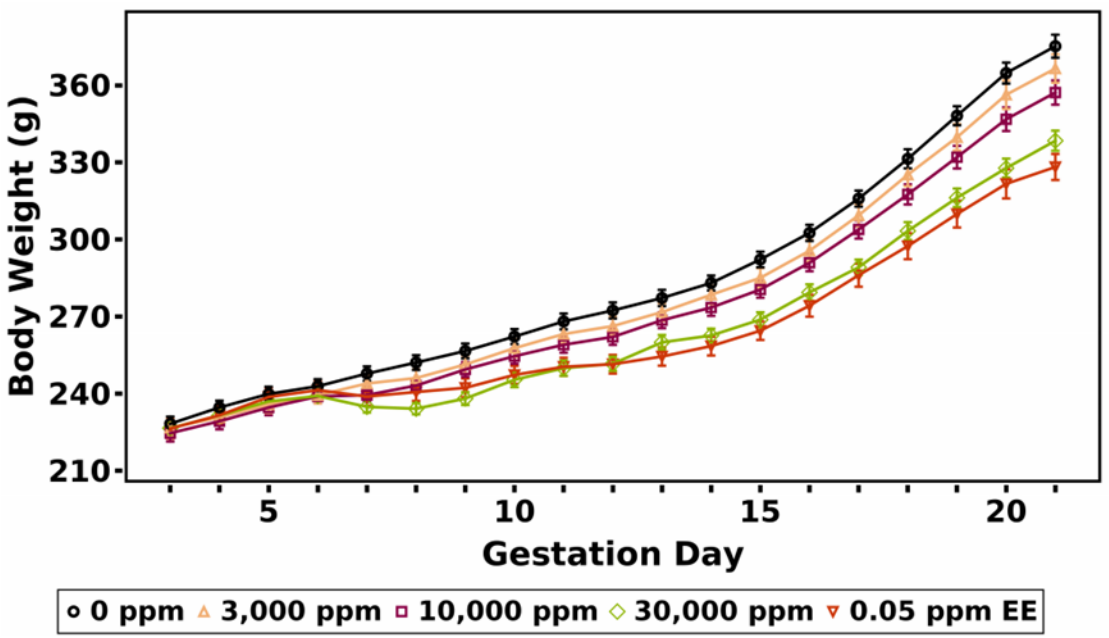
2H4MBP MOG Study Design

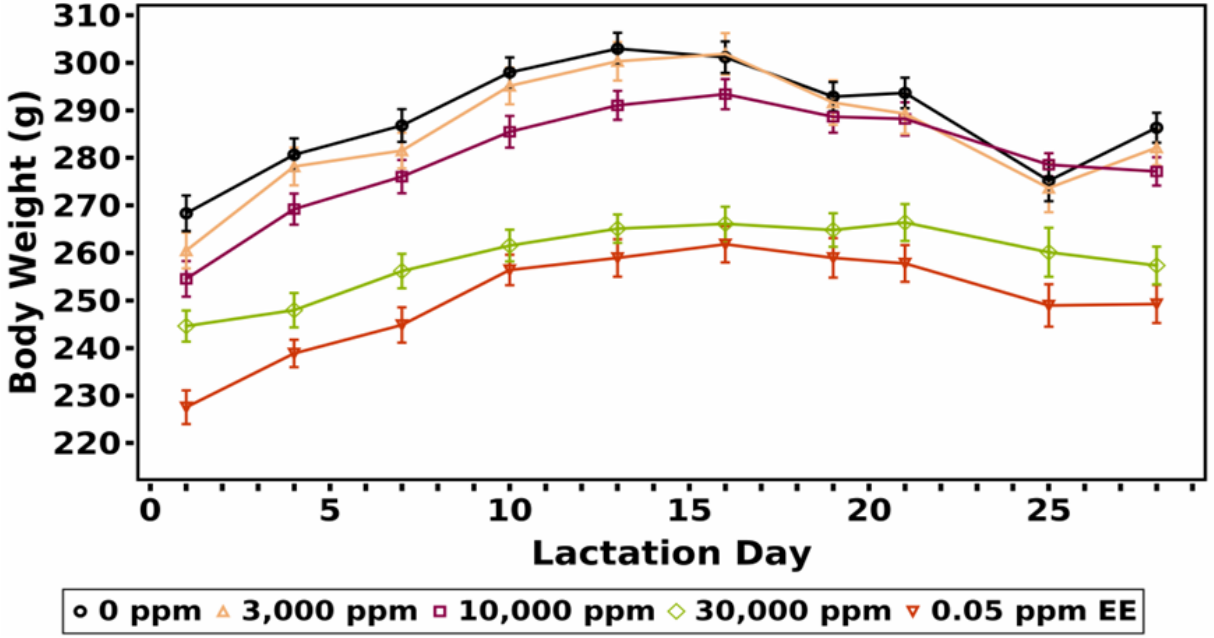


2H4MBP dose levels (dietary):
0, 3000, 10000, 30000 ppm

EE reference estrogen (dietary):
0.05 ppm

F_0 Dam Body Weights and Feed Consumption

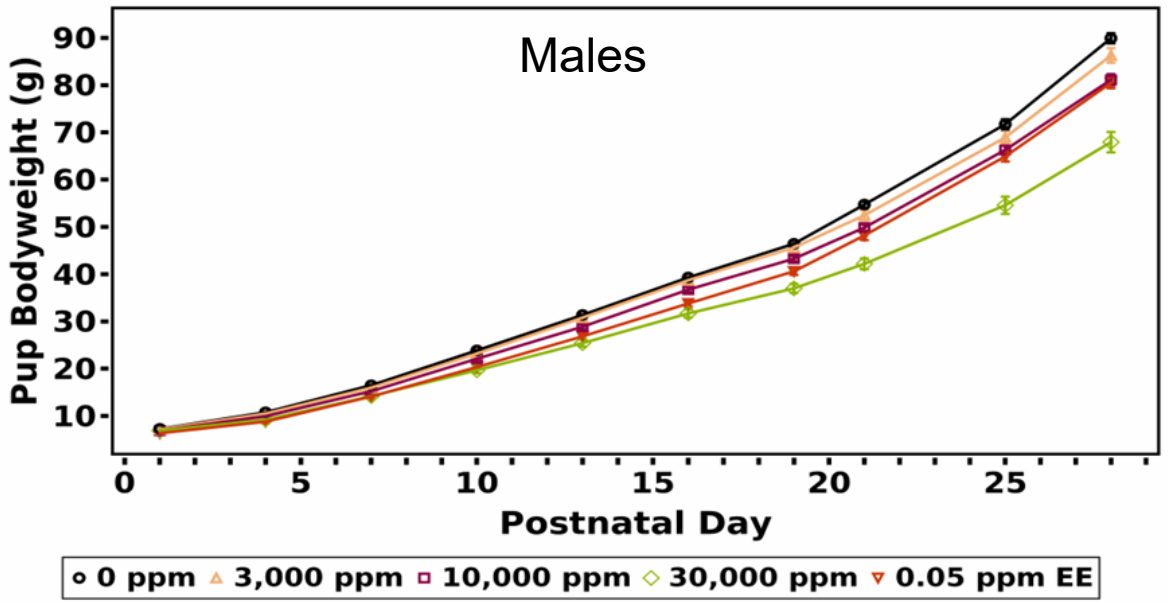


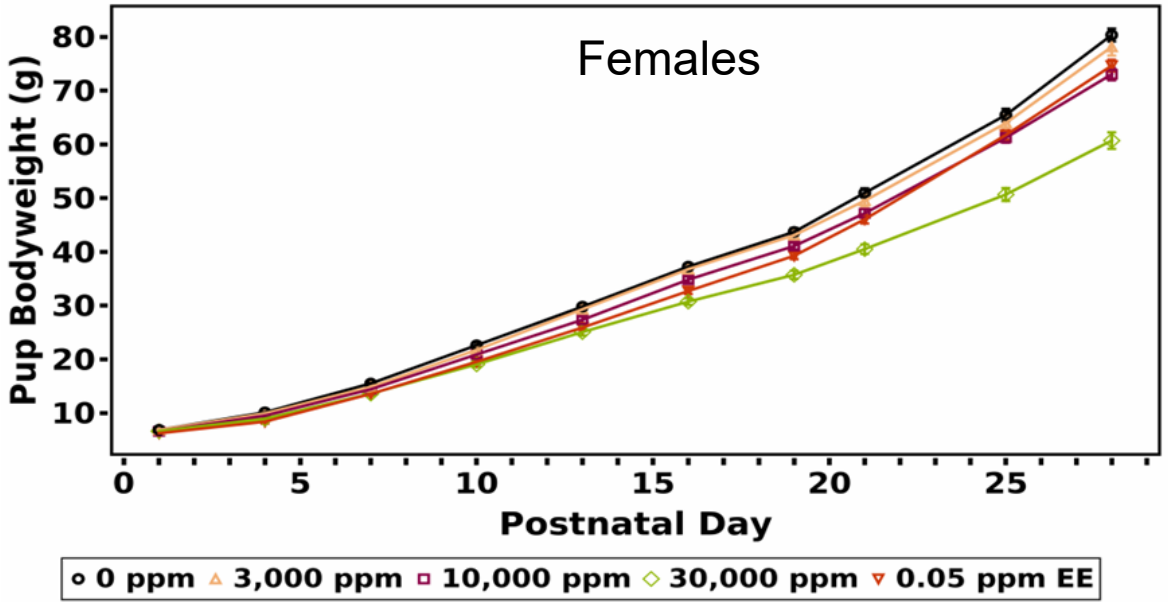


- Lower relative body weights in the 30000 and EE exposure groups
- No effects on feed consumption, litter size, or pup viability

	Chemical Intake (mg/kg/day)				
	0 ppm	**3000 ppm**	**10000 ppm**	**30000 ppm**	**EE 0.05 ppm**
GD 6-21	0.0 ± 0.0	204.5 ± 2.7	697.3 ± 15.4	2,644 ± 109.2	3.8 ± 0.2
LD 1-13	0.0 ± 0.0	484.1 ± 8.9	1,590.7 ± 29.6	5119.8 ± 216.3	8.4 ± 0.4

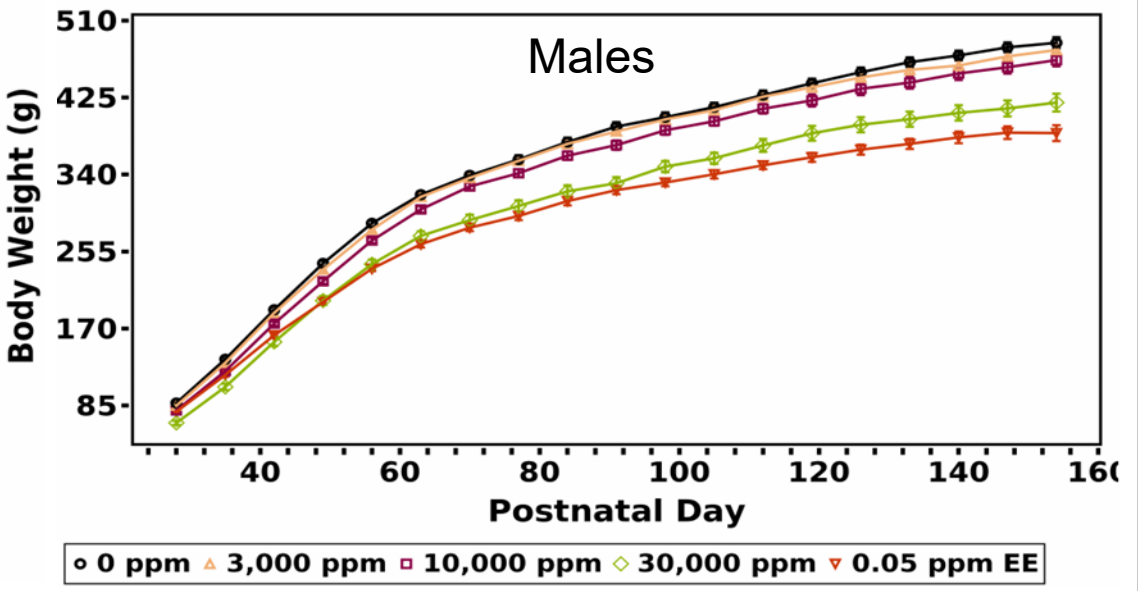
F_1 Pup Body Weights

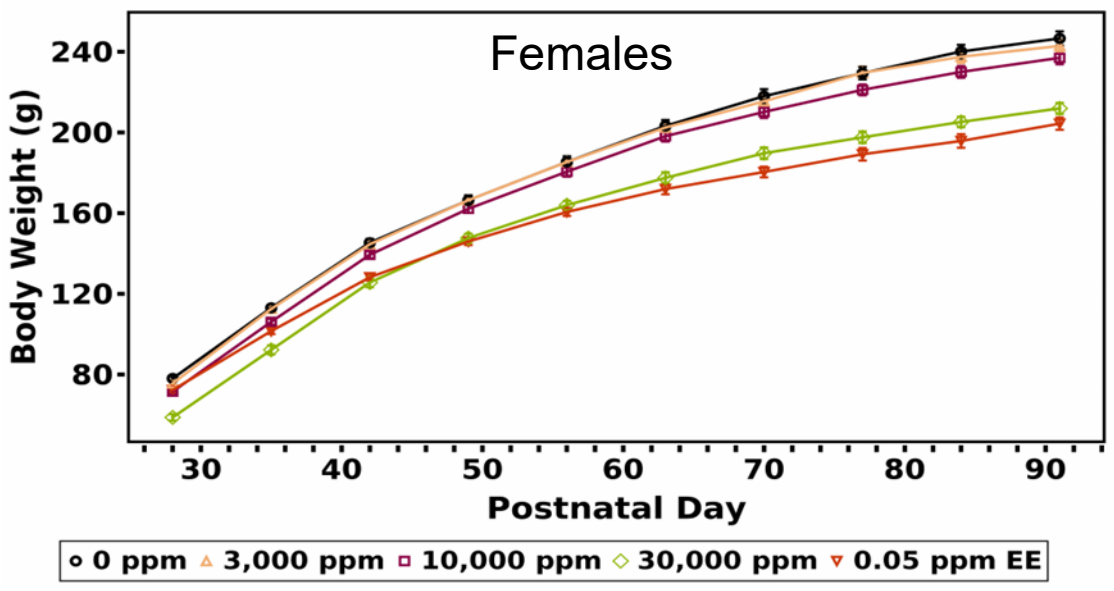




- Lower relative pup body weights in the 10000 ppm, 30000 ppm, and EE exposure groups

F_1 Postweaning Body Weights and Feed Consumption





- On PND 91, male body weights in the 10000 and 30000 ppm groups were 5% and 16% lower than the control group
- The EE group was 18% lower than the control group
- No effect on feed consumption

- On PND 91, female body weights in the 30000 ppm group were 14% lower than the control group
- The 10000 ppm group displayed transiently lower body weights (<10%)
- The EE group was 17% lower than the control group
- No effect on feed consumption

	Chemical Intake (mg/kg/day)				
	0 ppm	3000 ppm	10000 ppm	30000 ppm	EE 0.05 ppm
PND 28-91	0.0 ± 0.0	267.1 ± 3.9	947.9 ± 10.4	3002.5 ± 53.9	4.6 ± 0.1

	Chemical Intake (mg/kg/day)				
	0 ppm	3000 ppm	10000 ppm	30000 ppm	EE 0.05 ppm
PND 28-91	0.0 ± 0.0	286.5 ± 5.0	983.0 ± 15.3	3493.2 ± 65.5	5.4 ± 0.2

Magnitude of response and no indications of recovery support “Some Evidence” call.

F_1 Endocrine Sensitive Developmental Endpoints

- No effect on anogenital distance or male areola/nipple retention

Vaginal Opening	0 ppm	3000 ppm	10000 ppm	30000 ppm	EE 0.05 ppm
Litter Mean	35.3 ± 0.2**	35.4 ± 0.4	35.9 ± 0.3	38.1 ± 0.4**	24.3 ± 0.3**
Adjusted Mean	35.9 ± 0.2*	35.8 ± 0.3	35.9 ± 0.3	37.0 ± 0.3	24.3 ± 0.3**
BW on attainment (g)	115.7 ± 1.9**	114.3 ± 1.6	111.5 ± 1.6	109.0 ± 1.9*	59.0 ± 1.5**
BW at weaning (g)	80.6 ± 1.1**	78.1 ± 1.8	73.6 ± 1.3**	60.7 ± 1.6**	74.5 ± 1.2**

* Statistically significant $P \leq 0.05$
** Statistically significant $P \leq 0.01$

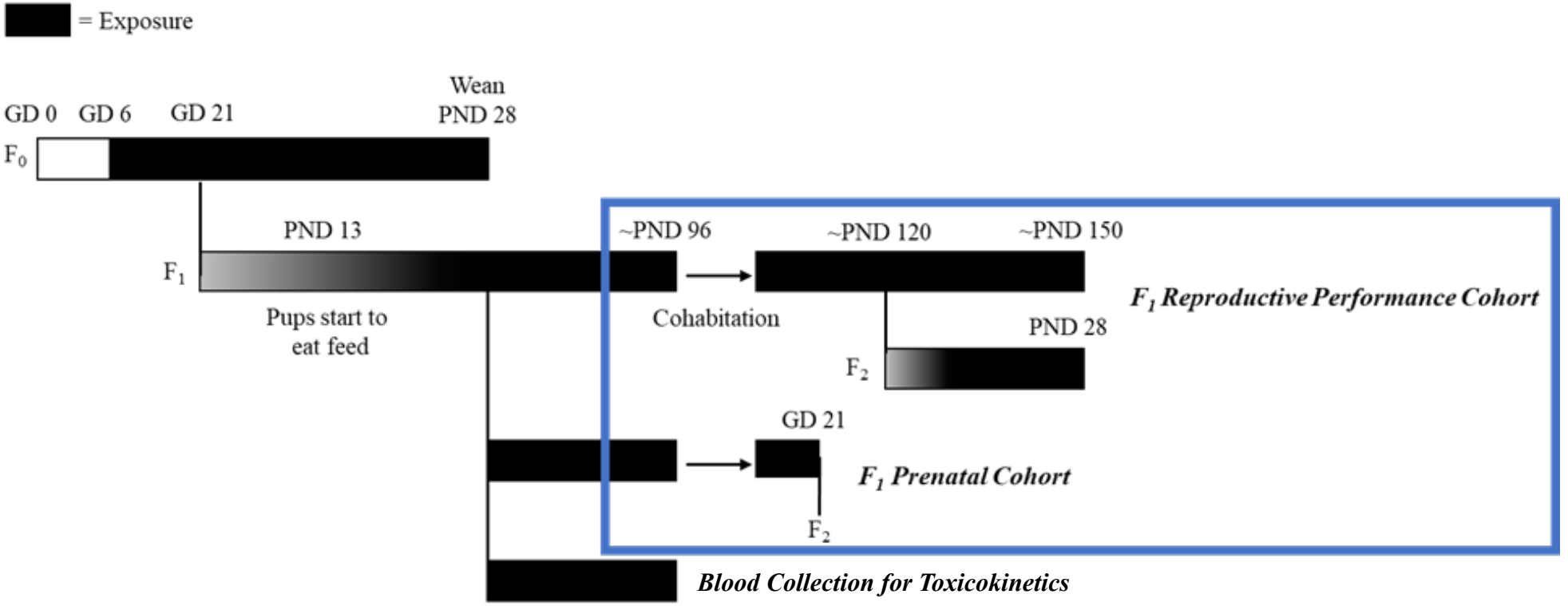
30000 ppm group weighed ~18% less on PND 35

Balanopreputial Separation	0 ppm	3000 ppm	10000 ppm	30000 ppm	EE 0.05 ppm
Litter Mean	43.7 ± 0.3**	44.0 ± 0.4	44.9 ± 0.3*	47.1 ± 0.4**	45.8 ± 0.3**
Adjusted Mean	44.7 ± 0.3	44.7 ± 0.3	44.8 ± 0.3	45.4 ± 0.3	44.8 ± 0.3
BW on attainment (g)	204.4 ± 2.9**	203.3 ± 2.9	196.4 ± 2.2	192.1 ± 2.8**	184.7 ± 2.2**
BW at weaning (g)	90.1 ± 1.1**	87.4 ± 1.6	81.4 ± 1.2**	68.6 ± 1.9**	80.3 ± 1.2**

* Statistically significant $P \leq 0.05$
** Statistically significant $P \leq 0.01$

30000 ppm group weighed ~18% lower on PND 42

F_1 Adult Cohorts



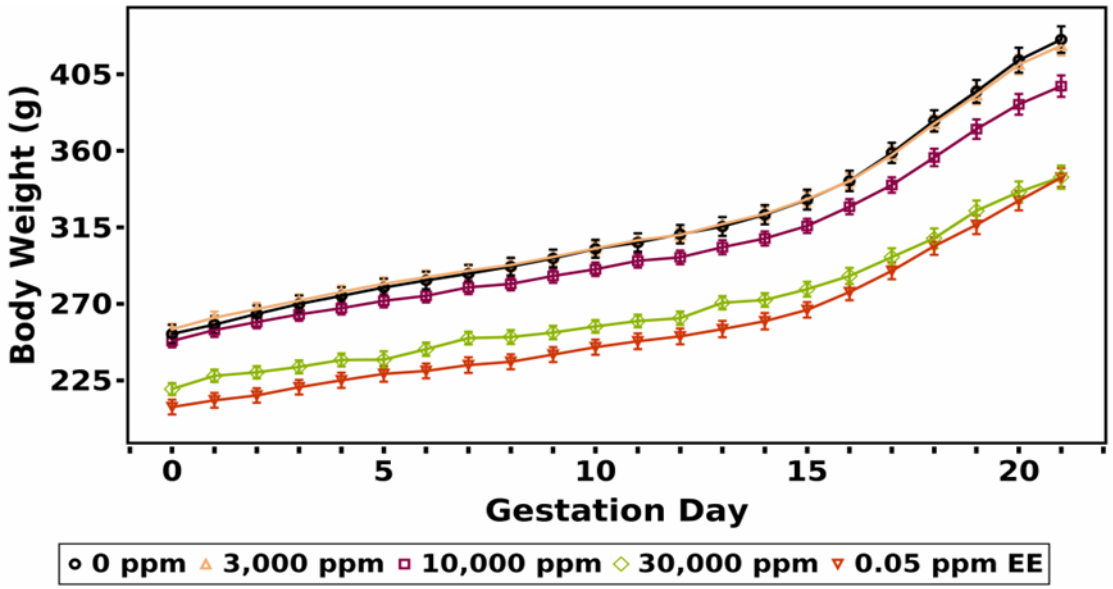
- No effects on viability or clinical observations

F_1 Vaginal Cytology, Andrology, and Reproductive Performance

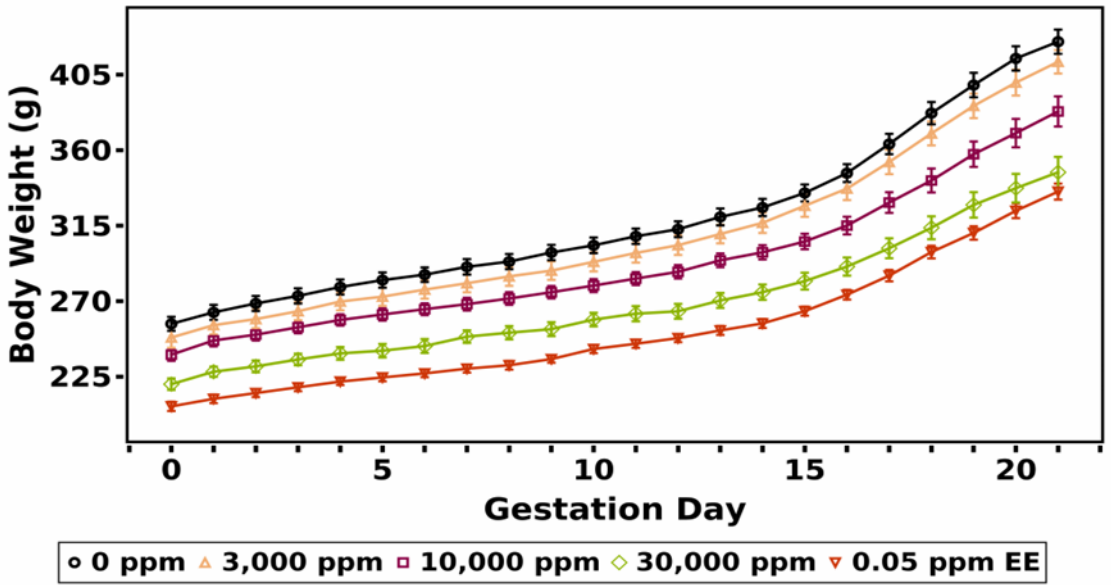
- Rats in the 10000 and 30000 ppm 2H4MBP groups in both cohorts displayed a higher probability of extended estrus and spent approximately 5% more time in estrus than did the control group
- Rats exposed to EE displayed a higher probability of extended diestrus. There were no 2H4MBP or EE exposure-related changes in estrous cycle length or number of cycles.
- Males in the 30000 ppm 2H4MBP group displayed lower cauda epididymal sperm counts (approximately 14%) and epididymis weight (approximately 6%) relative to control animals
- No effects on mating or fertility in either cohort

F_1 Gestational Body Weights

Reproductive Performance Cohort



Prenatal Cohort



- Feed consumption was similar amongst all 2H4MBP exposure groups

	Chemical Intake (mg/kg/day)									
	0 ppm		3000 ppm		10000 ppm		30000 ppm		EE 0.05 ppm	
	RPC	PC	RPC	PC	RPC	PC	RPC	PC	RPC	PC
GD 0-21	0.0 ± 0.0	0.0 ± 0.0	252.8 ± 6.3	224.2 ± 5.0	859.7 ± 23.2	791.8 ± 25.2	2844.2 ± 79.2	2684.4 ± 107.5	4.4± 0.2	4.6± 0.3

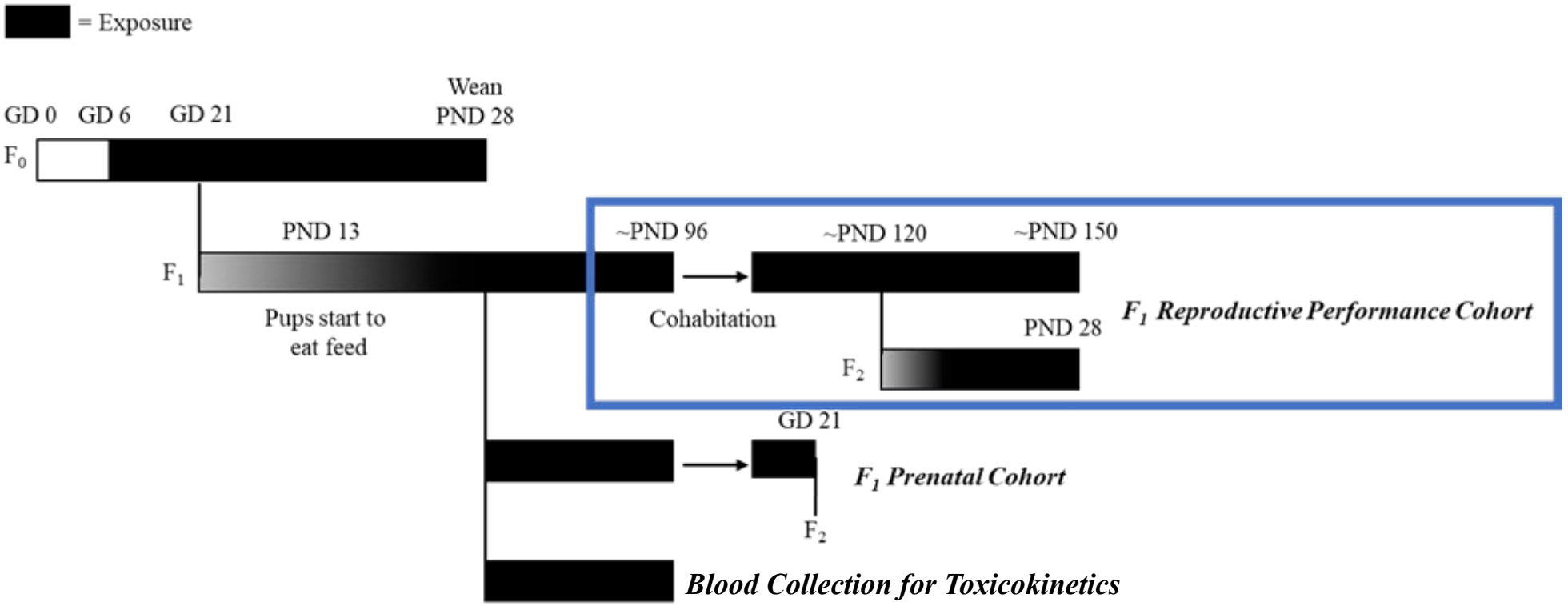
F_1 Prenatal Cohort Findings

- Fewer corpora lutea/dam in the 30000 ppm and EE exposure groups (~3 and 4, respectively), resulting in slightly smaller litter size, and lower gravid uterine weights, in the presence of lower relative body weights

  Magnitude of response supportive of the "Equivocal Evidence" call

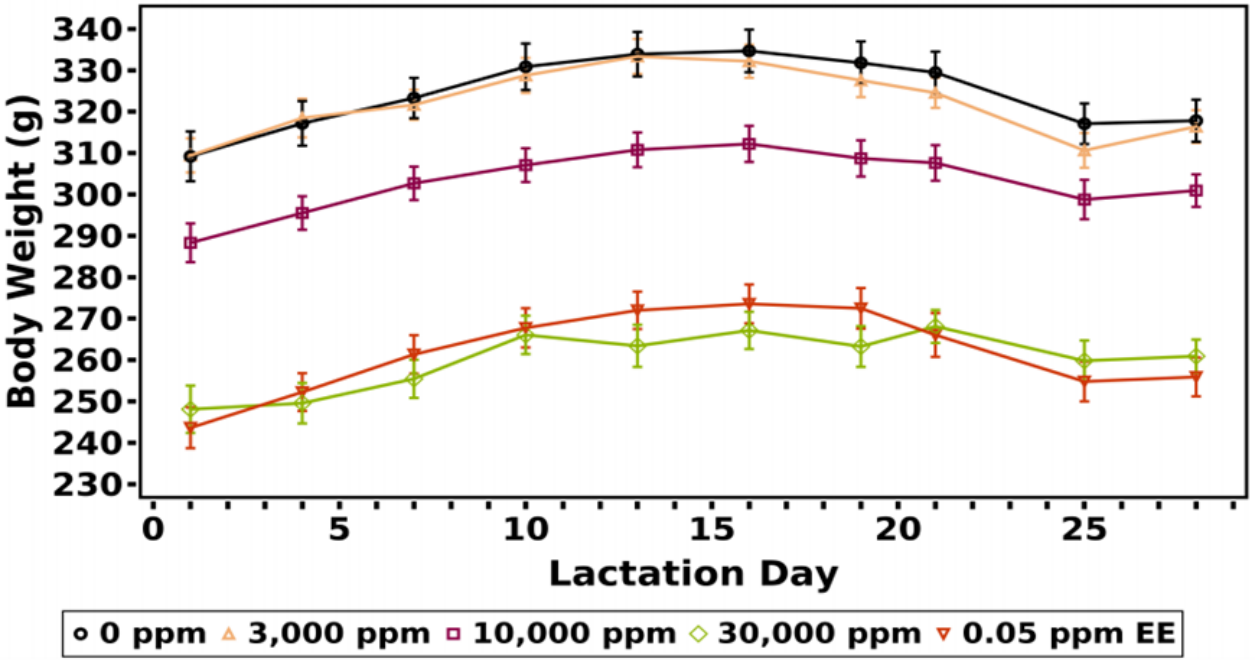
- No 2H4MBP-related effects on:
  - Postimplantation loss
  - Fetal weight
  - Fetal external, visceral, head or skeletal morphology

F_1 Reproductive Performance Cohort Findings



F_1 Reproductive Performance, Lactation Body Weights, and Feed Consumption

- No effects on reproductive performance
- Body weights were lower and consistent with respective premating weights
- Feed consumption in all 2H4MBP exposure groups were similar to control

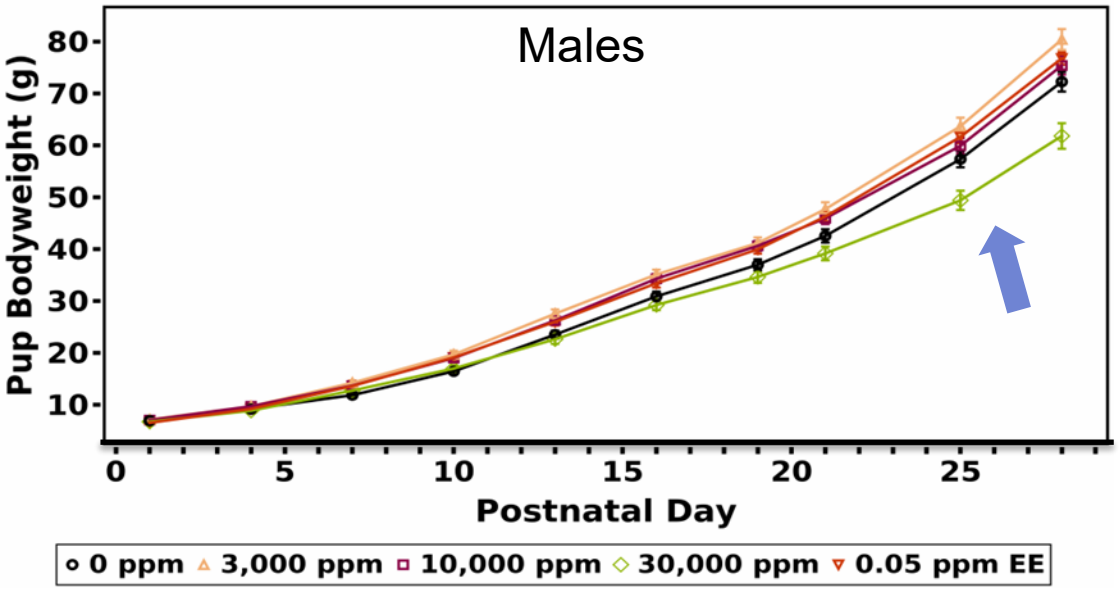


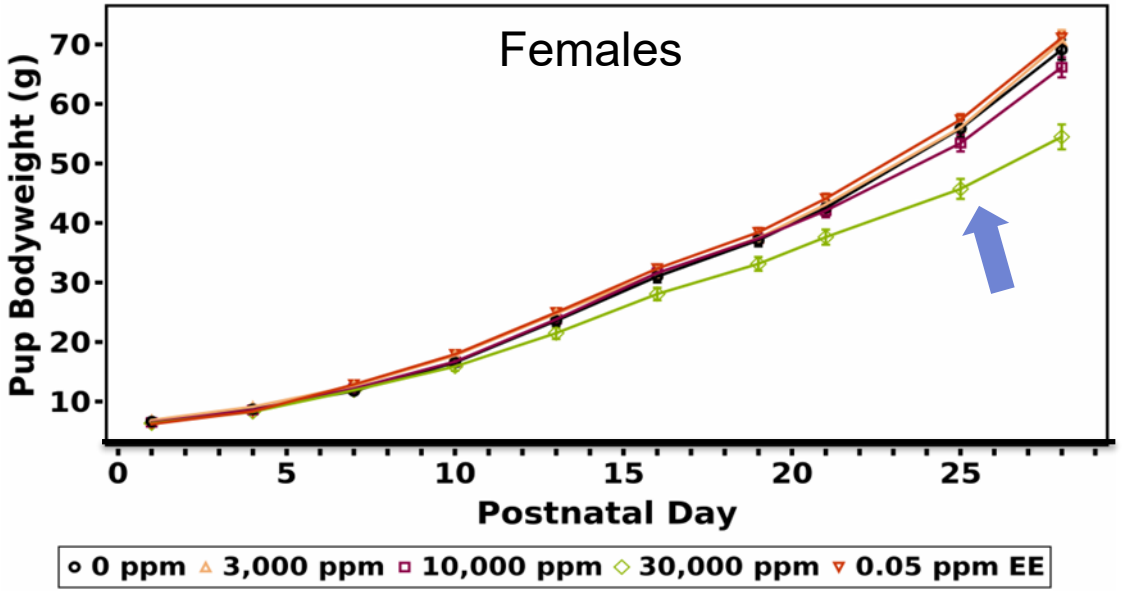
	Chemical Intake (mg/kg/day)				
	0 ppm	**3000 ppm**	**10000 ppm**	**30000 ppm**	**EE 0.05 ppm**
LD 1 - 13	0.0 ± 0.0	426.2 ± 13.5	1620.8 ± 60.0	5944.0 ± 268.8	8.9 ± 0.4

F_2 Viability

- The 30000 ppm and EE groups displayed a smaller litter size than control, consistent with what was observed in the prenatal cohort
- Lower body weights
- F_2 pups on PND 28 had kidney findings consistent with what was observed in adults

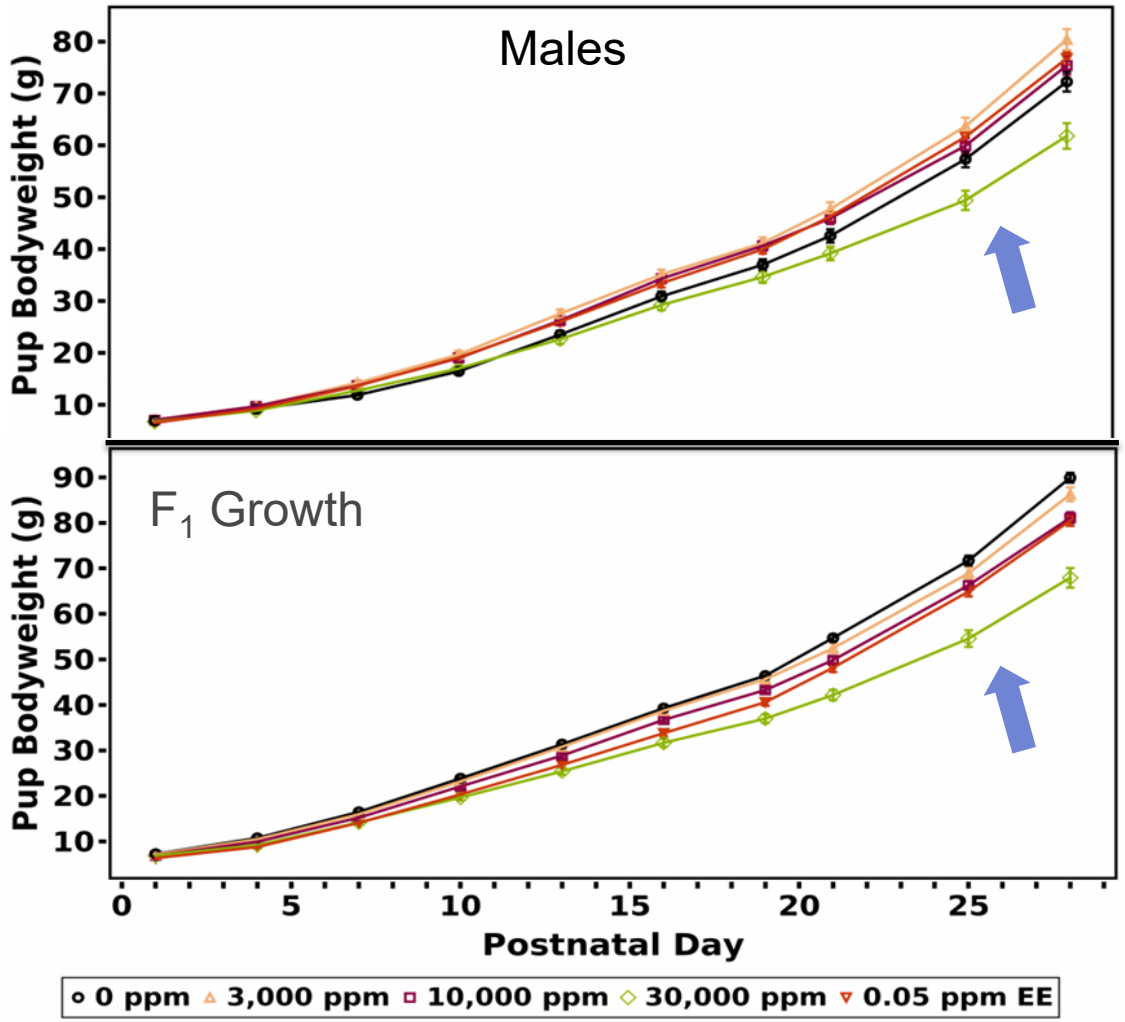
F_2 Growth

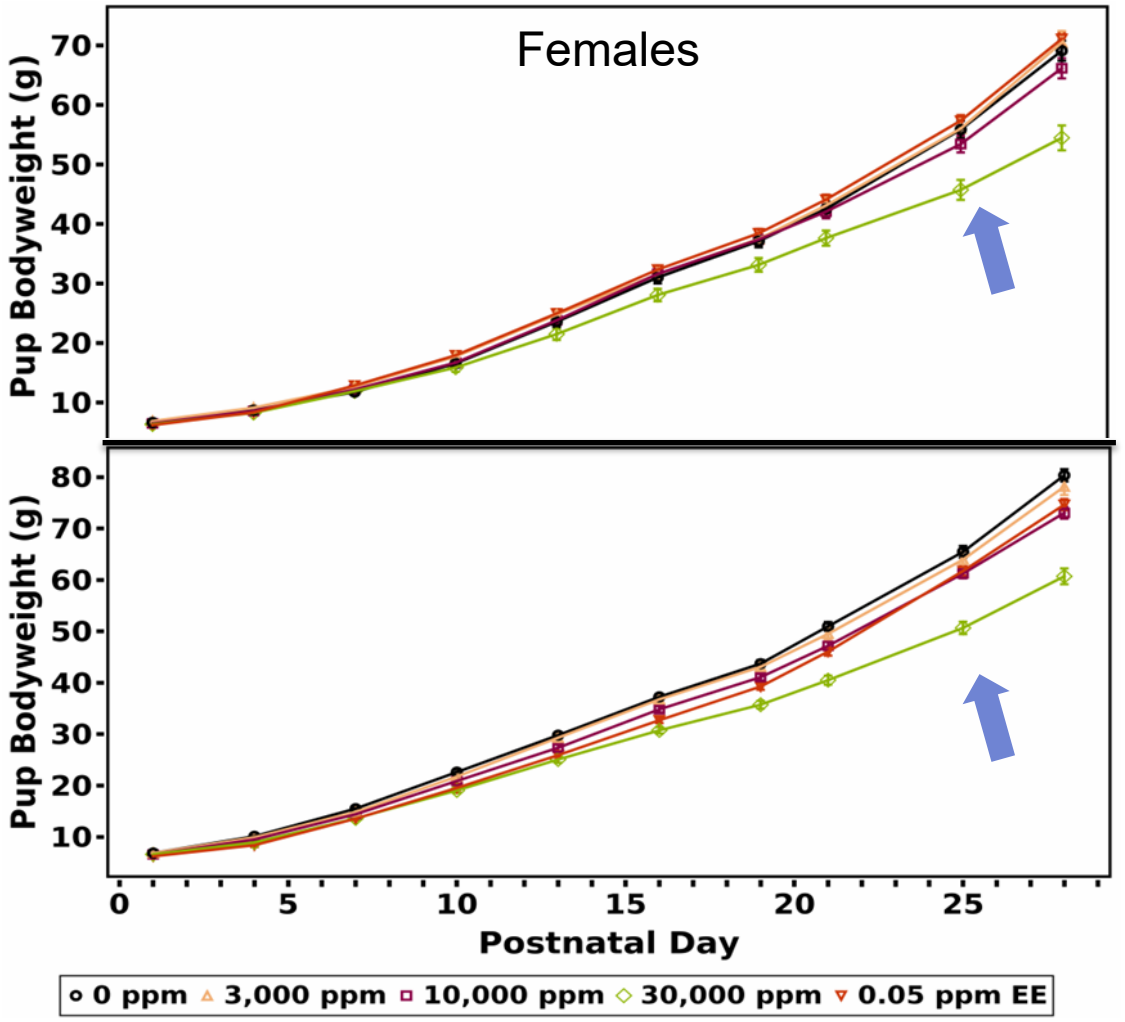




- Body weight response similar to what was observed in the F_1 generation

F_2 Growth





- Body weight response similar to what was observed in the F_1 generation

F_1 Male Pathology

- Gross Pathology and Tissue Weights
  - Rats in the 30000 ppm exposure group displayed a higher incidence of discolored (pale or dark) or enlarged kidneys and discolored (brown) urinary bladders. These findings correlated with histopathological changes consistent with a retrograde nephropathy. Similar findings were observed in females.
  - Male rats in all 2H4MBP-exposed groups in both cohorts displayed higher absolute and relative liver weights
  - The 30000 ppm exposure group in both cohorts displayed slightly lower testis, epididymis, and absolute ventral prostate weights, with a greater response in the reproductive performance cohort, likely due to the longer exposure period. There were no 2H4MBP-related histopathological findings.
  - One male in the 30000 ppm 2H4MBP group displayed a hypospadias; another displayed bilateral smaller testes
  - The 30000 ppm exposure group in both cohorts displayed lower absolute levator ani/bulbocavernosus muscle weights; however, when adjusted for body weight, this difference was negligible

2H4MBP Kidney Findings: Male Reproductive Cohort as an Example

	0 ppm	3000 ppm	10000 ppm	30000 ppm
Males (Litters)	41 (22)	40 (20)	40 (21)	40 (20)
Renal Tubule				
Epithelium, regeneration	0**	0	0	33 (17)** [1.2]
Interstitium, inflammation, chronic active	0**	0	0	22 (14)** [1.7]
Concretion	0**	0	0	35 (19)** [1.4]
Dilation	0**	0	0	37 (20)** [1.5]
Pelvis				
Concretion	0**	0	0	17 (13)** [1.5]
Urothelium				
Hyperplasia, total	0**	1 (1) [1.0]	0	18 (15)** [1.3]
Ulcer	0**	0	0	12 (9)** [1.0]
Papilla				
Necrosis	0**	0	0	10 (10)** [1.3]

F_1 Female Pathology

- Gross Pathology and Tissue Weights
  - Females exposed to 10000 and 30000 ppm 2H4MBP in both cohorts displayed lower absolute right and left ovarian weights. Females in the reproductive performance cohort exposed to 30000 ppm 2H4MBP displayed lower absolute adrenal gland weights
  - Both cohorts of the EE group had lower absolute ovarian and adrenal cortical weights

Plasma 2H4MBP and Metabolite Levels in PND 28 and PND 56 Pups

- 2H4MBP formed several Phase 1 and Phase 2 metabolites
- Concentrations of free (unconjugated) and total (free and conjugated) analytes increased with increasing exposure concentrations
- Total analyte concentrations were significantly higher than free concentrations demonstrating extensive conjugation
- Free and total 2H4MBP concentrations were similar or higher than those of other Phase 1 metabolites
- There was no apparent sex difference

A comparison of 2H4MBP plasma concentrations in the current study with human plasma concentrations following repeated dermal exposure to 2H4MBP showed that rat concentrations were within 4-fold of the human concentrations

- *Mutlu et al (2017); DOI: 10.1093/jat/bkx070*

Conclusions

- Under the conditions of this modified one-generation (MOG) study, there was ***equivocal evidence of reproductive toxicity*** of 2-hydroxy-4-methoxybenzophenone (2H4MBP) in Hsd:Sprague Dawley® SD® rats based on a decrease in F_2 litter size in both the prenatal and reproductive performance cohorts.
- Under the conditions of this MOG study, there was ***some evidence of developmental toxicity*** of 2H4MBP in Hsd:Sprague Dawley® SD® rats based on the observed postnatal growth retardation. The relationship of the increased occurrence of diaphragmatic and hepatodiaphragmatic hernias in F_1 adults and F_2 pups to 2H4MBP exposure is unclear.
- Exposure to 2H4MBP was not associated with signals consistent with alterations in estrogenic, androgenic, or antiandrogenic action. Exposure to 2H4MBP was associated with lower F_1 and F_2 mean body weights; this effect on body weight contributed to the apparent 2H4MBP-related decreases in male reproductive organ weights. Mating and littering were not significantly affected by 2H4MBP exposure. Exposure to 2H4MBP was associated with nonneoplastic kidney lesions in the F_0, F_1, and F_2 generations. Expected estrogenic responses were observed in the EE group.

Questions?