NTP
National Toxicology Program

Draft NTP Developmental and Reproductive Toxicity Technical Report on the Modified One-Generation Study of 2-Ethylhexyl *p*-Methoxycinnamate (EHMC) (CASRN 5466-77-3) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats with Prenatal, Reproductive Performance, and Subchronic Assessments in F_1 Offspring

DART Report 06

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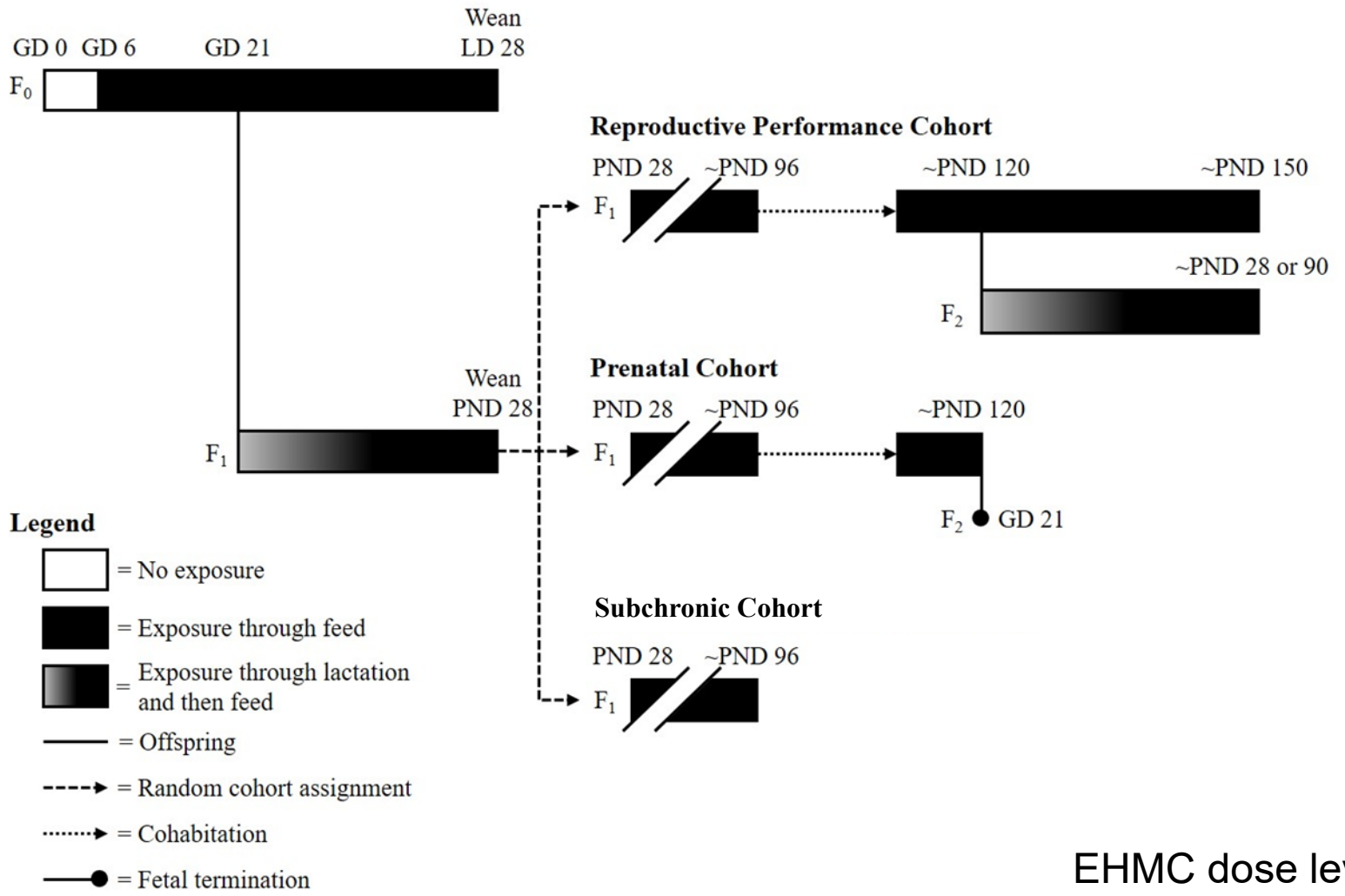
EHMC MOG Study Design

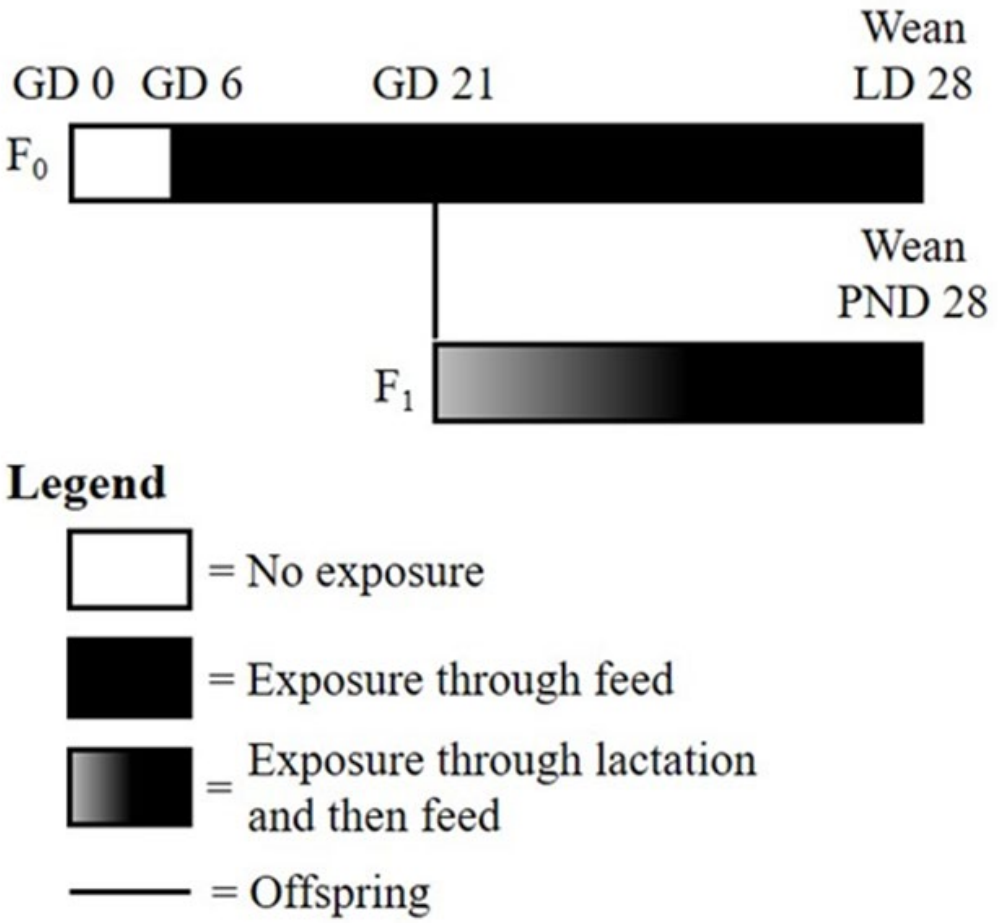


EHMC dose levels (dietary):
0, 1000, 3000, 6000 ppm

EHMC Dose Range-Finding Study Design

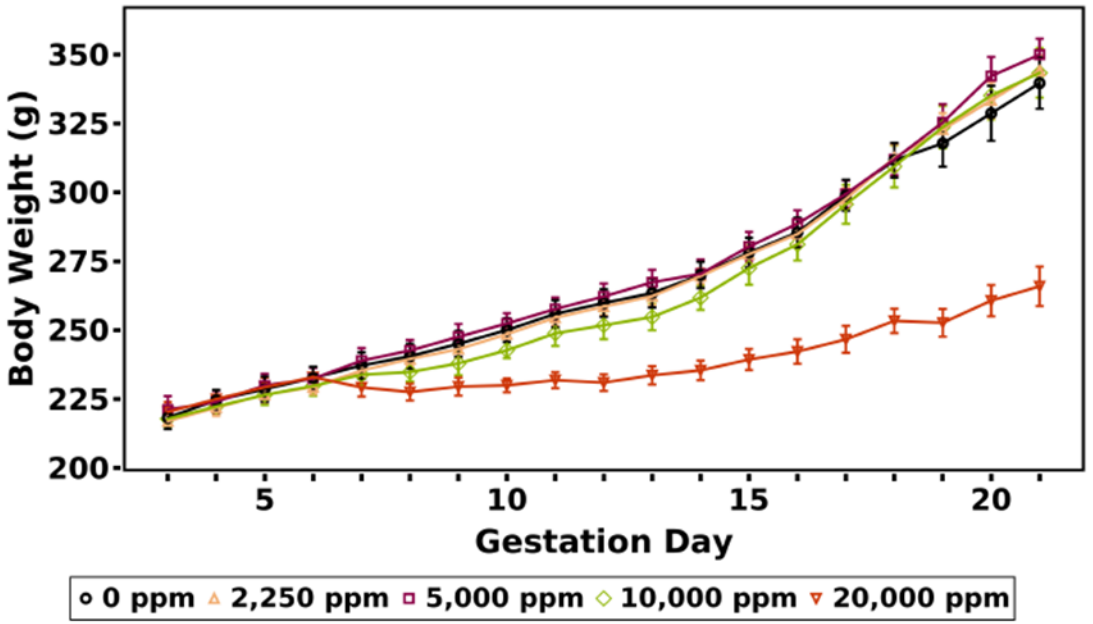
N = 8-13 time-mated dams

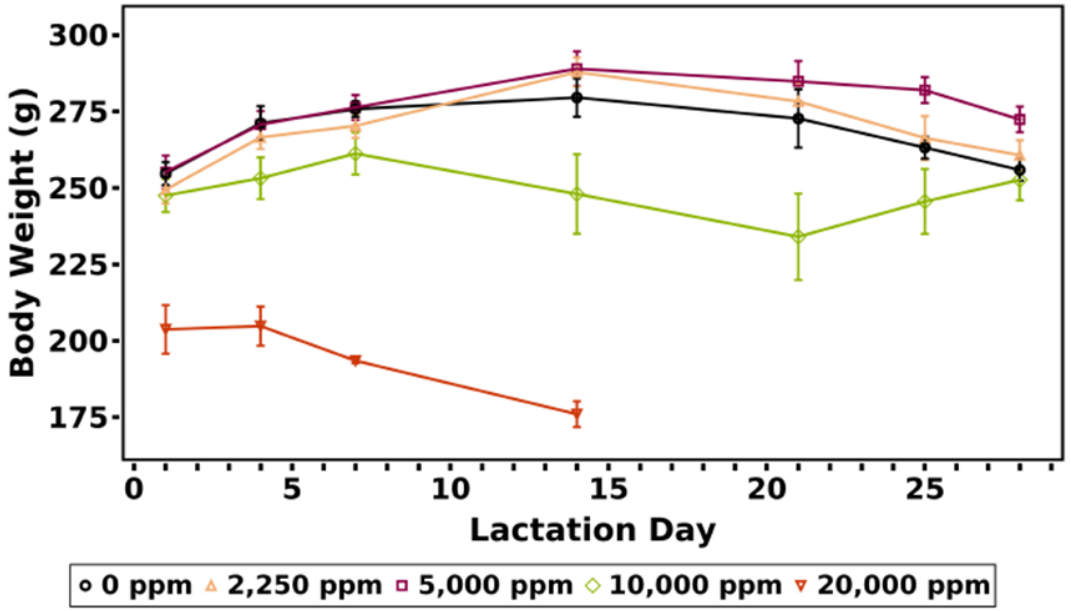
Extra dams were added for bioanalytical assay development



EHMC Dose Range-Finding Study Results

F_0 Dam Body Weights



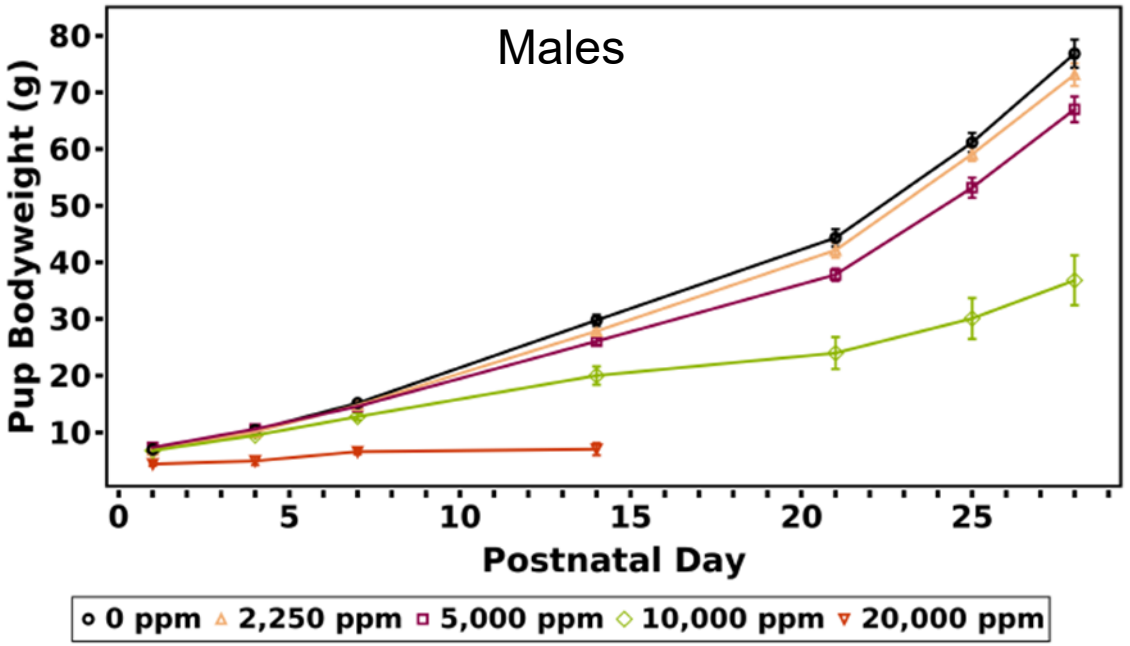


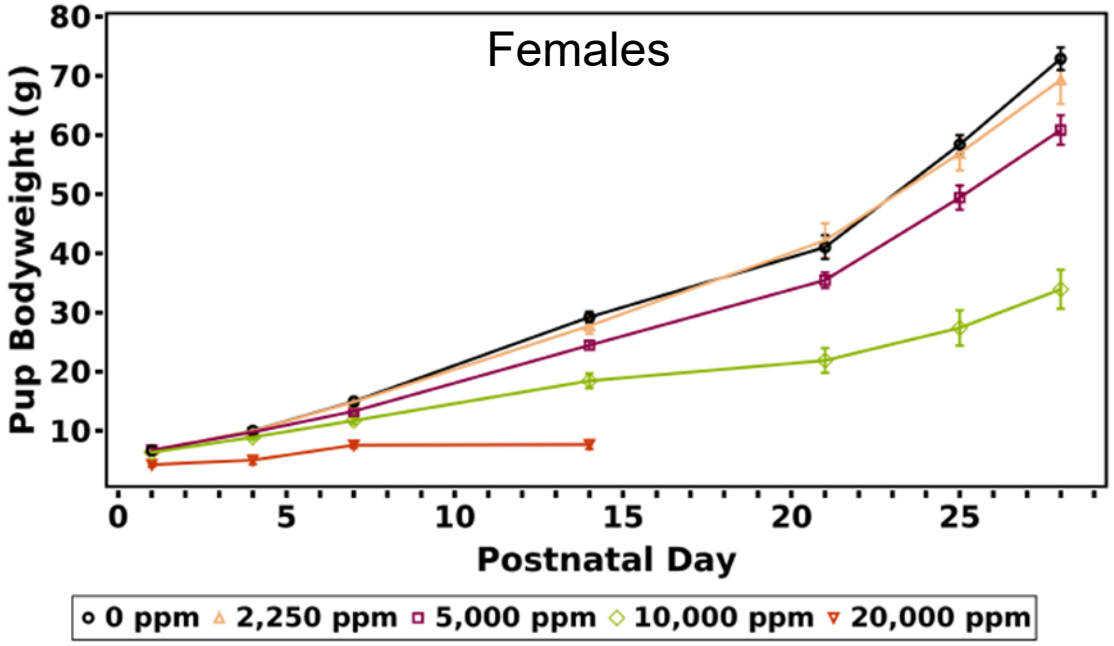
- Lower body weights in the 20000 ppm exposure group concomitant with lower feed consumption
- Removal of the 20000 ppm exposure group on PND 14 (pup viability)

	Chemical Intake (mg/kg/day)				
	0 ppm	**2250 ppm**	**5000 ppm**	**10000 ppm**	**20000 ppm**
GD 6 - 21	0.0 ± 0.0	161.1 ± 3.5	365.2 ± 10.1	713.5 ± 29.0	1,841.4 ± 125.7
LD 1 - 14	0.0 ± 0.0	409.8 ± 31.1	924.9 ± 14.1	1,615 ± 125.6	-

EHMC Dose Range-Finding Study Results

F_1 Pup Body Weights and Viability



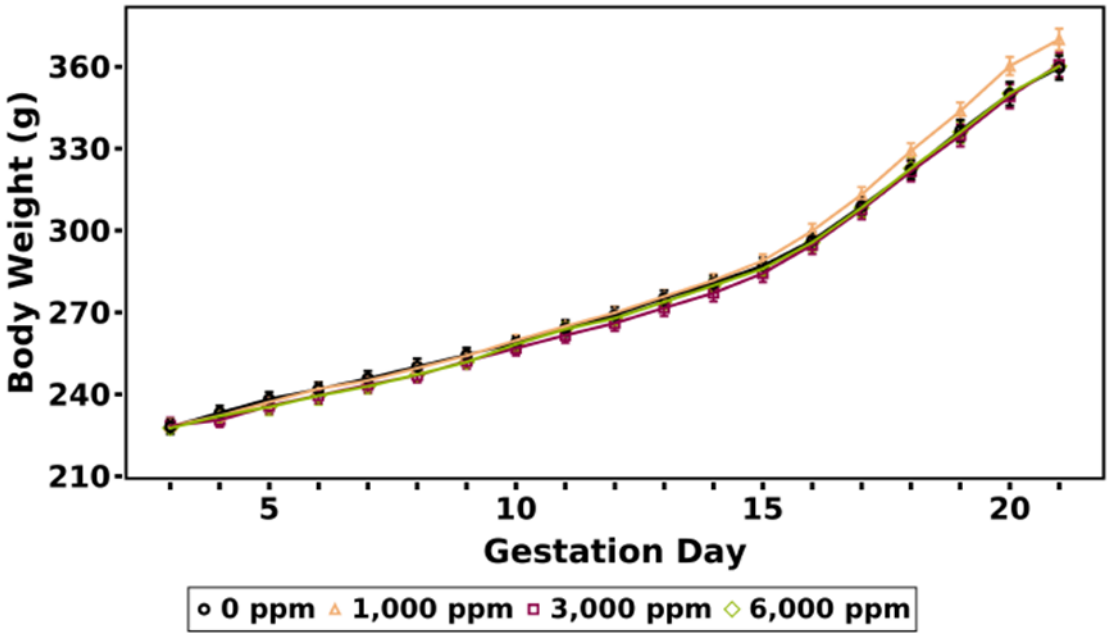


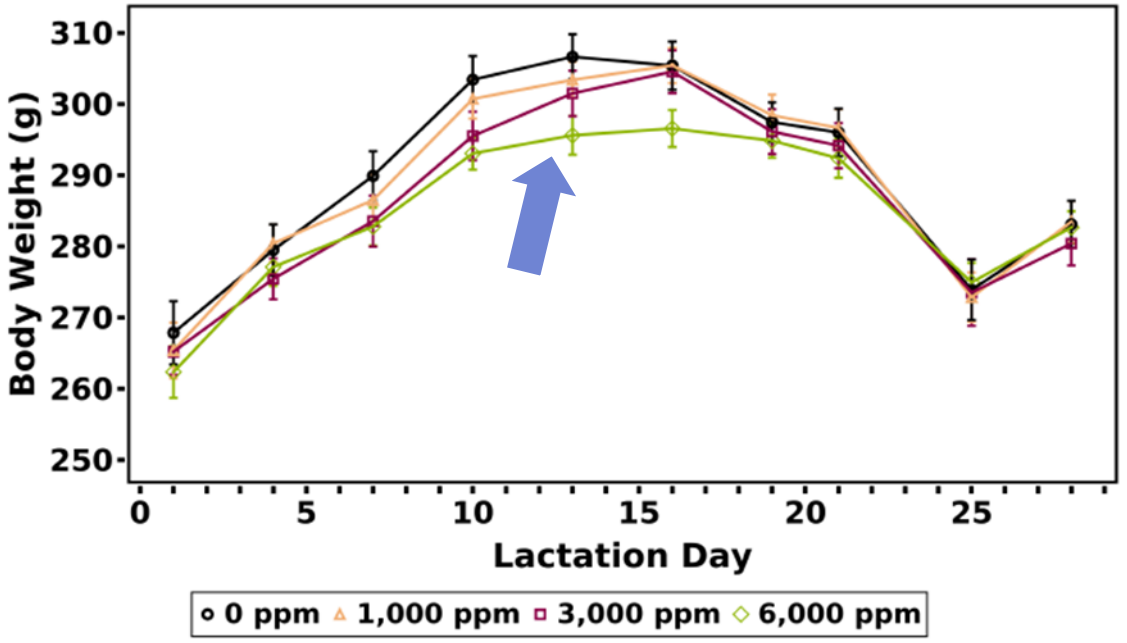
- No effects on gestation length or live litter size
- Lower pup body weights in the 10000 and 20000 ppm exposure groups
- Decrease in number of live pups/litter in the 20000 ppm exposure group

Exposure Concentration Selection Rationale

- Selection of 6000 ppm as the highest exposure concentration for the modified one-generation study was based on excessively lower pup mean body weight observed at 10000 ppm in the dose range-finding study
- On PND 28, relative to control, pup body weights from dams exposed to 5000 ppm were lower for both females (17%, statistically significant) and males (13%), approximating the targeted 10% reduction to ensure a challenge recognizing the limited sample size
- Exposure concentrations and spacing for the MOG study (1000, 3000, and 6000 ppm) were selected to identify a no-observed-adverse-effect level and to avoid excessive overlap of the ingested doses due to increased feed consumption during pregnancy and lactation

F_0 Dam Body Weights and Feed Consumption

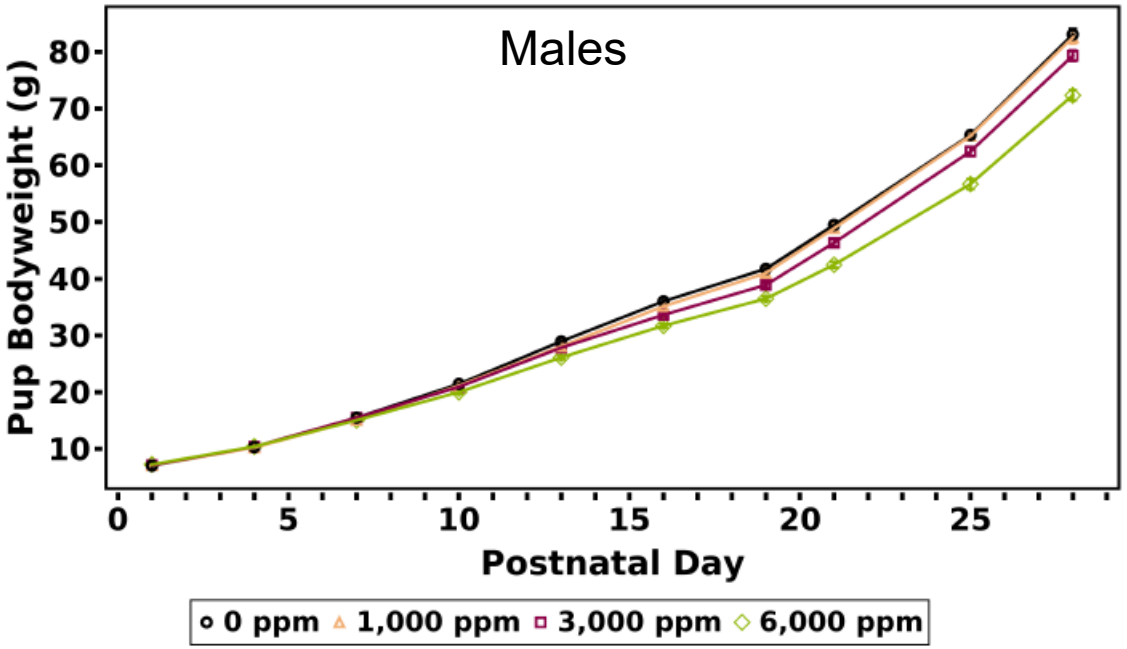


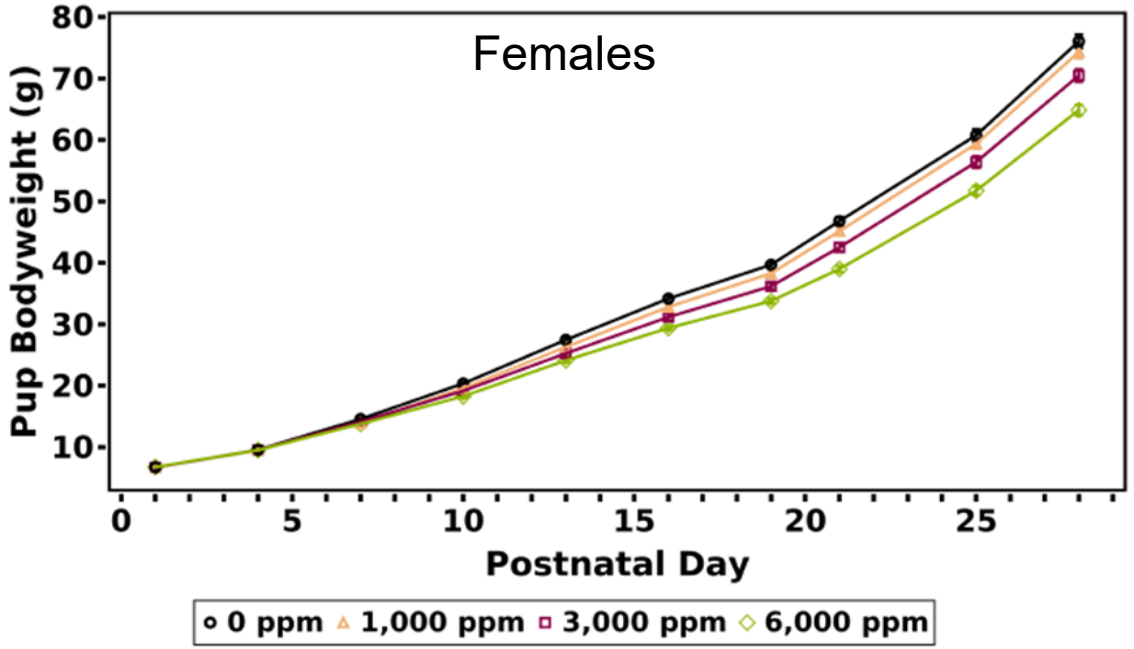


- No effects on feed consumption, reproductive performance, litter size, or pup viability

	Chemical Intake (mg/kg/day)			
	0 ppm	**1000 ppm**	**3000 ppm**	**6000 ppm**
GD 6 - 21	0.0 ± 0.0	69.6 ± 0.6	207.2 ± 3.4	418.7 ± 6.9
LD 1 - 13	0.0 ± 0.0	161.2 ± 2.7	474.8 ± 8.2	920.2 ± 24.2

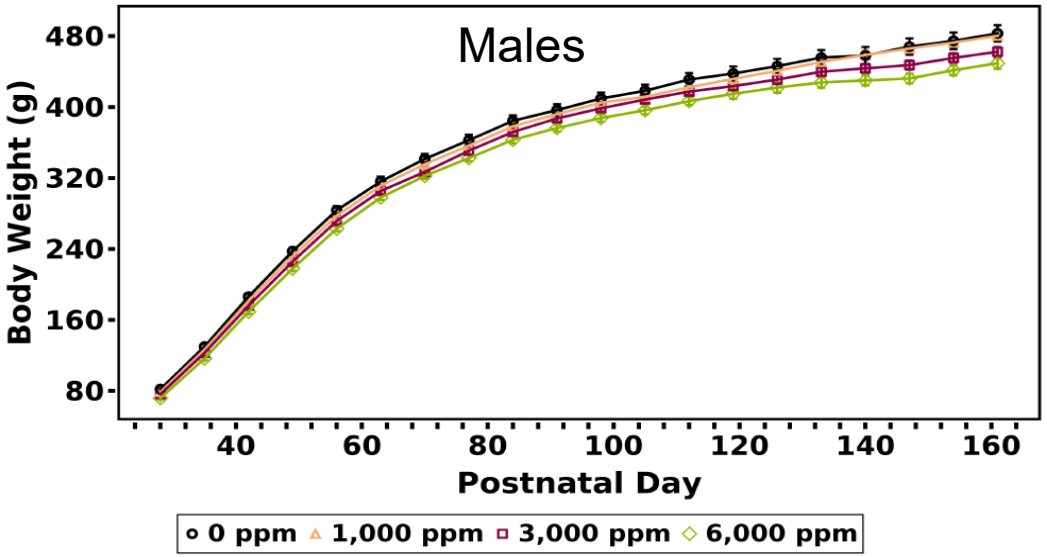
F_1 Pup Body Weights

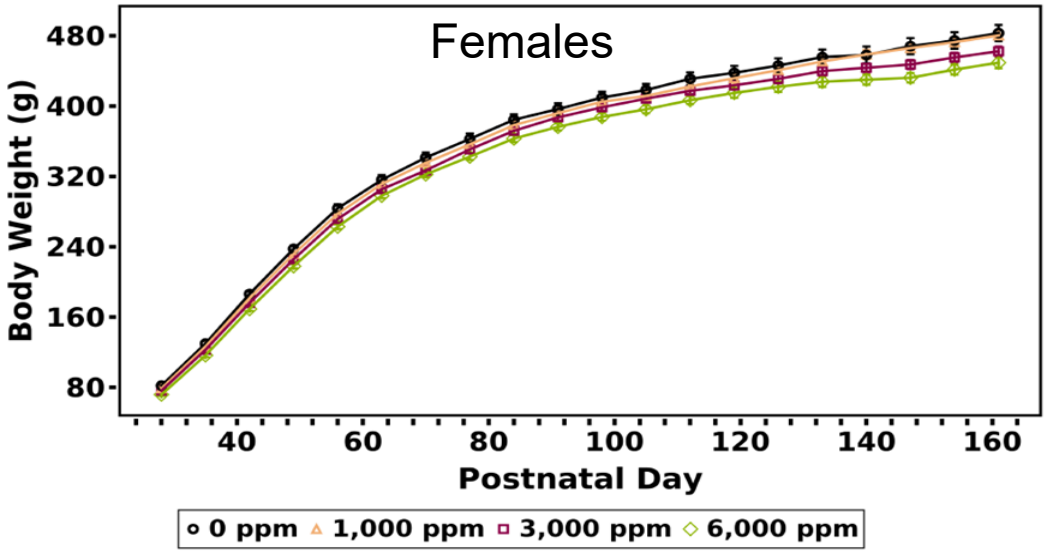




- Lower pup body weights in the 3000 and 6000 ppm exposure groups

F_1 Postweaning Body Weights and Feed Consumption





- Male body weights in the 6000 ppm group were 5-12% lower than the control group
- Minimal decreases in feed consumption in the 6000 ppm group over the PND 70-91 interval

	Chemical Intake (mg/kg/day)			
PND	**0 ppm**	**1000 ppm**	**3000 ppm**	**6000 ppm**
28-91	0.0 ± 0.0	79.9 ± 0.7	242.3 ± 2.3	491.4 ± 5.3

- Female body weights in the 6000 ppm group were 7-14% lower than the control group
- Female body weights in the 3000 ppm group were significantly lower (6–11%) relative to that of the control group until PND 56, after which the mean body weights were <5% lower
- No effects on feed consumption

	Chemical Intake (mg/kg/day)			
PND	**0 ppm**	**1000 ppm**	**3000 ppm**	**6000 ppm**
28-91	0.0 ± 0.0	87.0 ± 0.9	262.6 ± 2.7	528.1 ± 7.0

Magnitude of response and indications of recovery supportive of the “Equivocal” call.

F_1 Endocrine Sensitive Developmental Endpoints

- No effects on male areola/nipple retention

Vaginal Opening	0 ppm	1000 ppm	3000 ppm	6000 ppm
Litter Mean	34.1 ± 0.3**	35.0 ± 0.2	35.8 ± 0.4**	36.8 ± 0.3**
Adjusted Mean	34.4 ± 0.3**	35.1 ± 0.2	35.7 ± 0.3*	36.5 ± 0.3**
BW on attainment (g)	106.7 ± 2.0	107.3 ± 1.3	107.1 ± 1.4	107.7 ± 2.4
BW at weaning (g)	77.5 ± 1.8**	73.0 ± 1.1	69.4 ± 1.0**	66.1 ± 1.6**

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

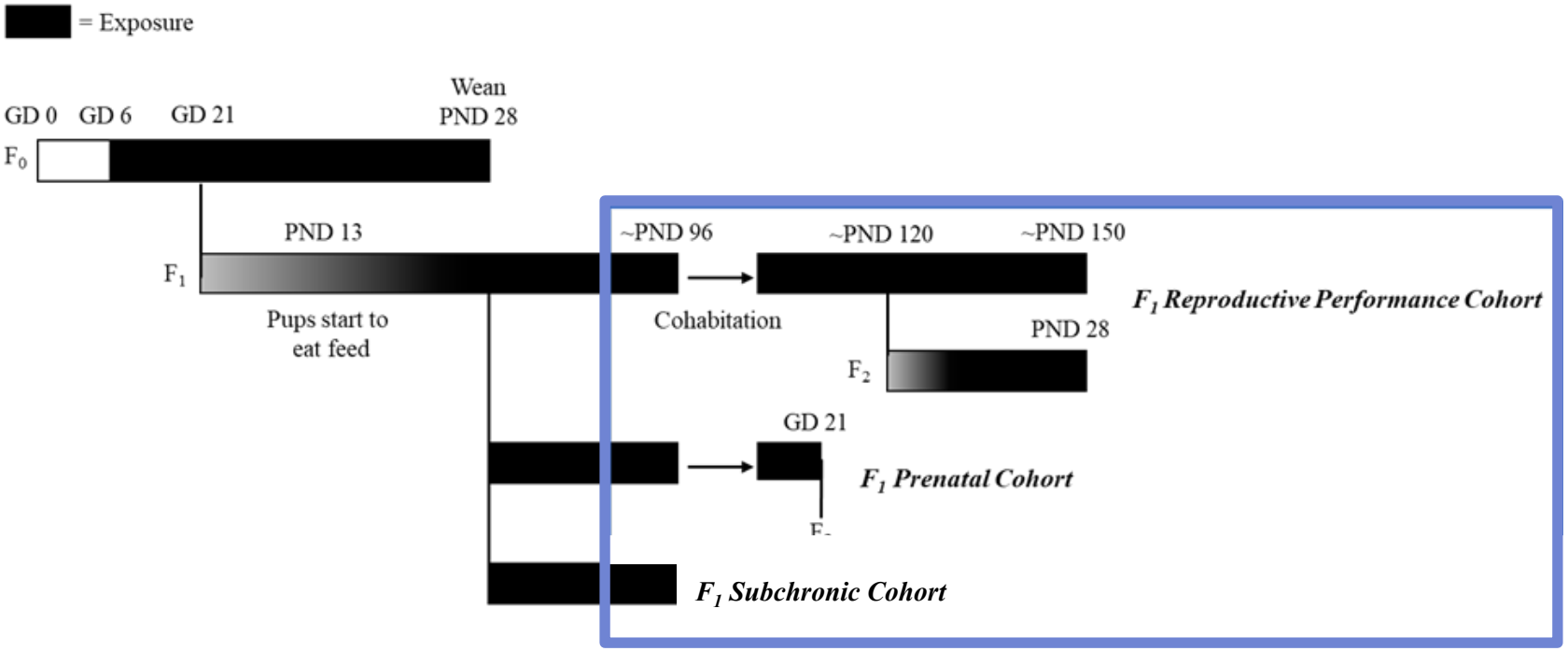
Balanopreputial Separation

Balanopreputial Separation	0 ppm	1000 ppm	3000 ppm	6000 ppm
Litter Mean	44.9 ± 0.3**	45.4 ± 0.6	45.3 ± 0.4	48.4 ± 0.6**
Adjusted Mean	45.6 ± 0.3**	45.6 ± 0.6	45.2 ± 0.3	47.8 ± 0.5**
BW on attainment (g)	207.9 ± 3.5	203.5 ± 4.0	199.2 ± 1.9	214.1 ± 3.4
BW at weaning (g)	84.5 ± 1.6**	80.9 ± 1.2	78.2 ± 0.9**	73.6 ± 1.5**

* Statistically significant $P \leq 0.05$

** Statistically significant $P \leq 0.01$

F_1 Adult Cohorts



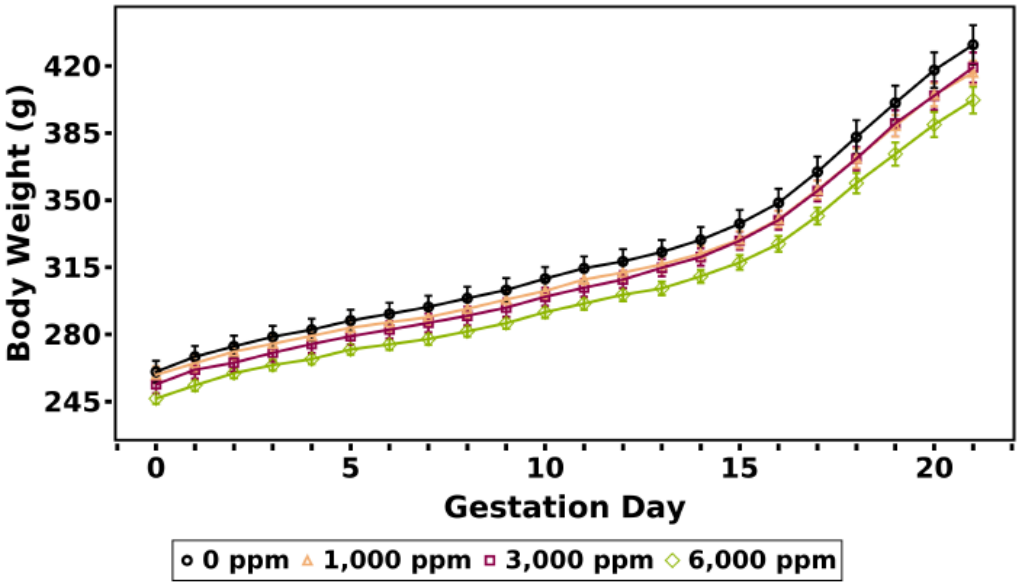
- No effects on viability or clinical observations

F_1 Vaginal Cytology, Andrology, and Reproductive Performance

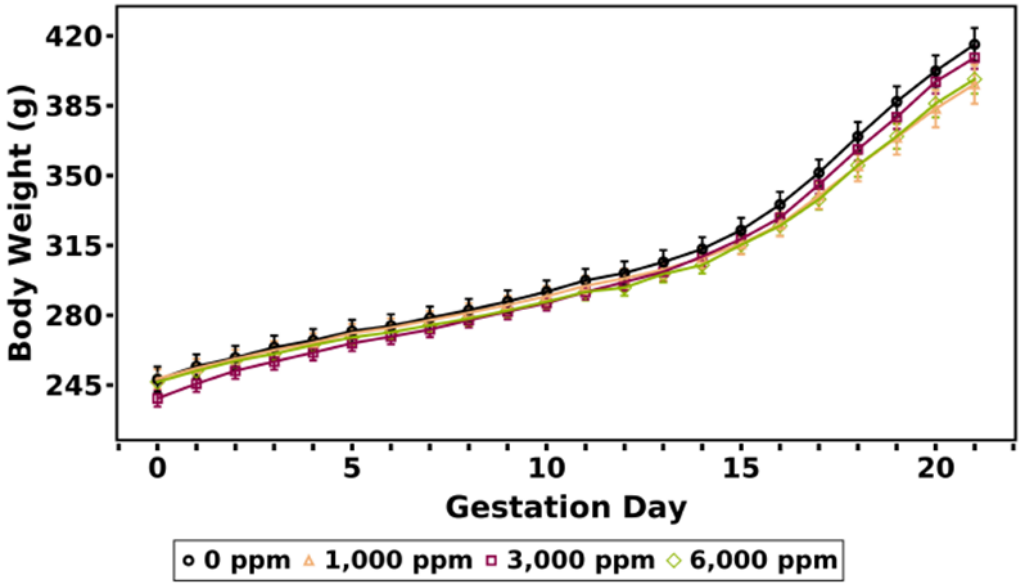
- Minimal effects on estrous cyclicity
  - Rats in the 6000 ppm EHMC exposure group from all three cohorts spent slightly more time in estrus compared to the control group (approximately 28% of the days versus approximately 20% in control)
  - In the reproductive performance cohort (which had the largest group size), all EHMC exposed groups displayed an increased probability of extended estrus, and decreased probability of extended diestrus
- There were no EHMC exposure-related changes in estrous cycle length or number of cycles
- No EHMC exposure-related effects on sperm parameters
- No effects on mating or fertility in either cohort

F_1 Gestational Body Weights

Reproductive Performance Cohort



Prenatal Cohort



- Lower body weights in the 6000 ppm exposure group
- Gestational feed consumption (g/animal/day) in the 6000 ppm exposure group was slightly lower during the GD 0–21 interval

	Chemical Intake (mg/kg/day)							
	0 ppm		1000 ppm		3000 ppm		6000 ppm	
	RPC	PC	RPC	PC	RPC	PC	RPC	PC
GD 0-21	0.0 ± 0.0	0.0 ± 0.0	73.2 ± 1.2	74.4 ± 1.3	220.5 ± 2.5	220.0 ± 3.9	435.1 ± 5.7	430.3 ± 5.4

F_1 Prenatal Cohort Findings

- No EHMC exposure-related effects on:
  - Number of implants
  - Postimplantation loss
  - Fetal weight
  - External, visceral, or head morphology

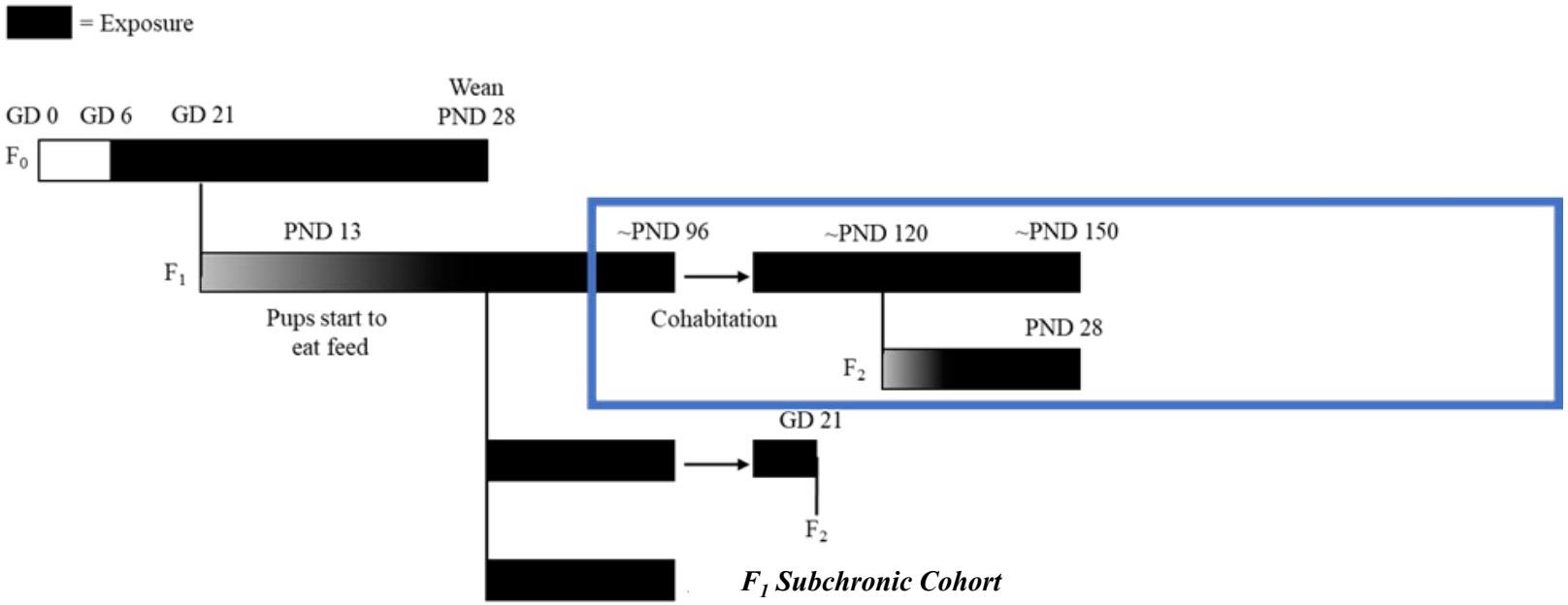
Fetal Skeletal Findings

	0 ppm	1000 ppm	3000 ppm	6000 ppm
No. Litters Examined	19	17	12	16
No. Fetuses Examined	283	211	183	218
Rib Lumbar 1, rudimentary; Unilateral or bilateral [V]				
Fetuses	12(4.24)**	8(3.79)	7(3.83)	22(10.09)**
Litters	5(26.32)	5(29.41)	2(16.67)	7(43.75)

** Statistically significant $P \leq 0.01$

- EHMC, upon metabolism, 2-ethylhexanol and 2-ethylhexanoic acid are formed
  - *Fennell et al (2017); DOI: 10.1080/00498254.2017.1400129*
- These metabolites have been shown to have teratogenic potential
  - Administration of 12.5 mM/kg of 2-ethylhexanol (approximately 1,680 g/kg) to Wistar rats on GD 12 was associated with hydronephrosis, tail, and limb malformations
  - Administration of 2-ethylhexanoic acid at the same mM dose induced a greater response for these endpoints in addition to inducing cardiovascular defects
    - *Ritter (1987); https://doi.org/10.1002/tera.1420350107*
- Given that this is a relatively common background variation, and that this finding was not consistent with the responses of the known metabolites (given separately), this was considered to be a spurious finding

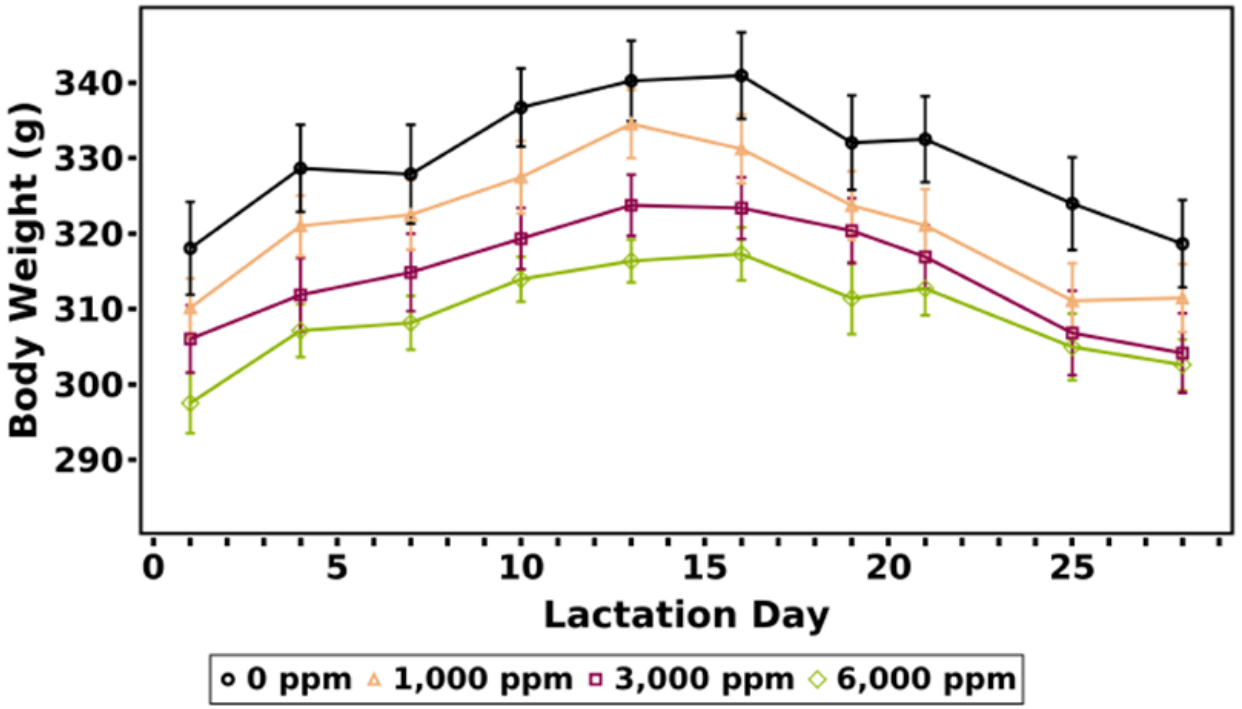
F_1 Reproductive Performance Cohort Findings



F_1 Subchronic Cohort

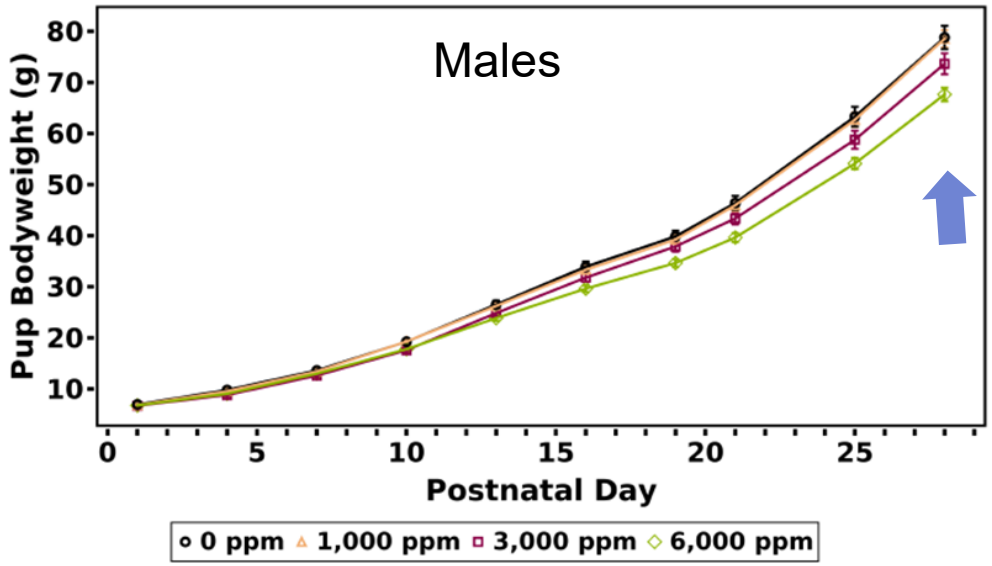
F_1 Reproductive Performance, Lactation Body Weights, and Feed Consumption

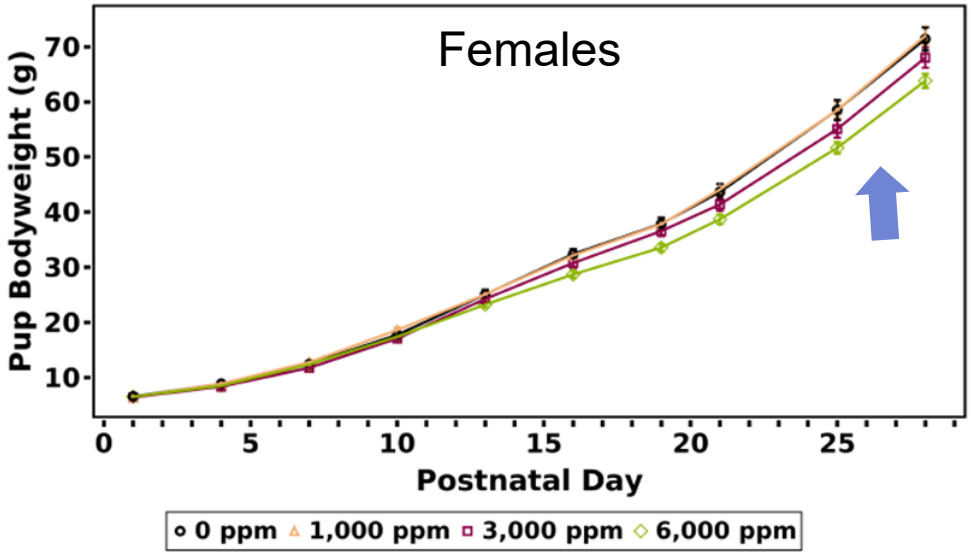
- No effects on reproductive performance
- Body weights were lower and consistent with respective premating weights
- Feed consumption in all EHMC exposure groups were similar to control



	Chemical Intake (mg/kg/day)			
	0 ppm	**1000 ppm**	**3000 ppm**	**6000 ppm**
LD 1 - 13	0.0 ± 0.0	138.5 ± 3.9	417.5 ± 19.2	842.4 ± 32.8

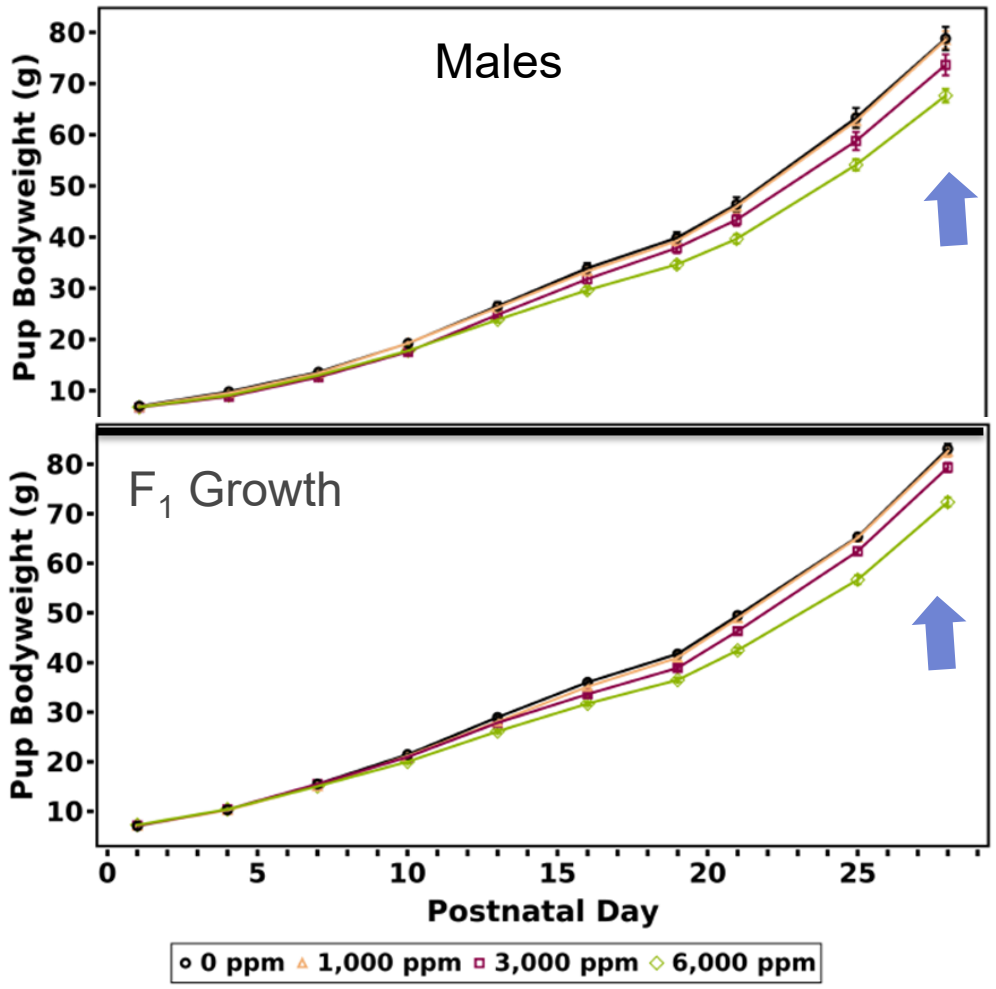
F_2 Growth

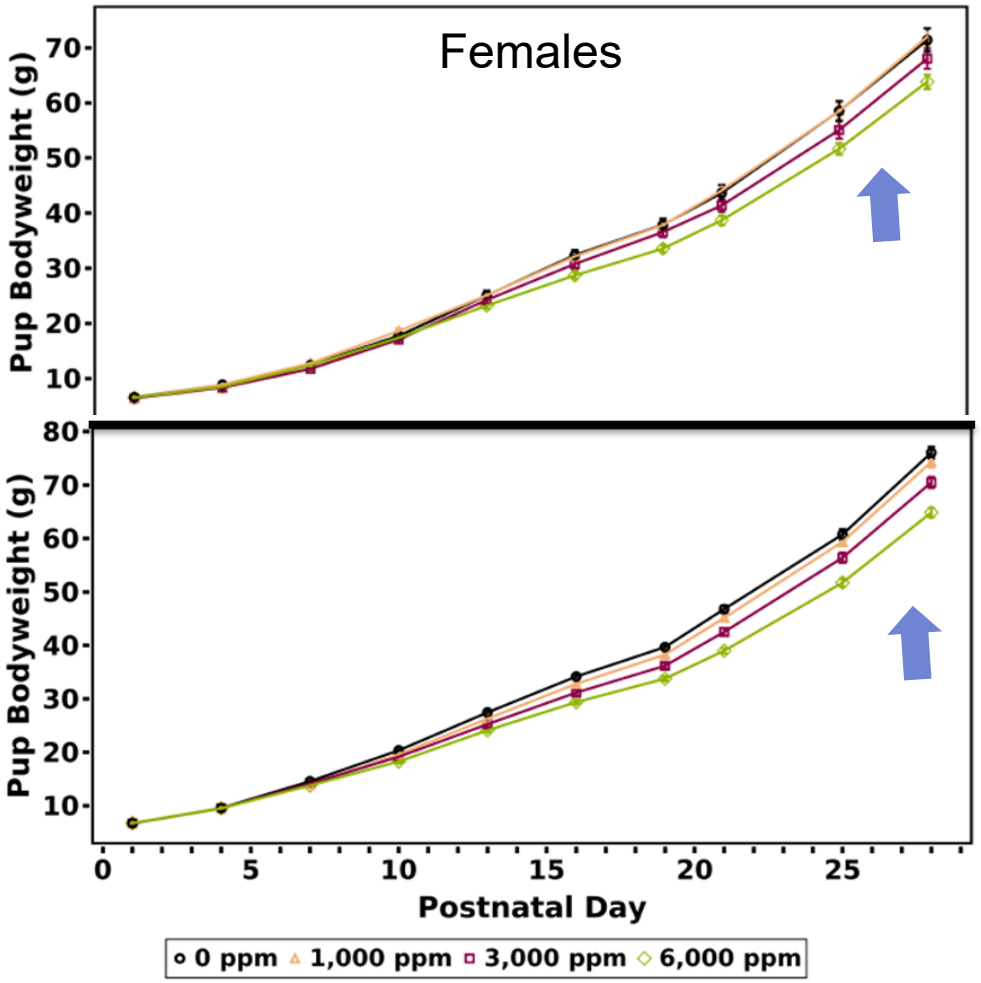




- No effects on F_2 viability

F_2 Growth





- No effects on F_2 viability
- Body weight response similar to what was observed in the F_1 generation

Pathology

- Gross Pathology
  - Exposed rats in all adult cohorts exposed to EHMC did not display any gross pathology findings attributable to EHMC exposure
- Clinical Pathology
  - Decrease in alanine aminotransferase (ALT) activity in the 3000 and 6000 ppm female rats; this was not toxicologically relevant.
- Histopathology
  - No histopathological findings in any of the cohorts were considered related to EHMC exposure

Conclusions

- Under the conditions of this modified one-generation (MOG) study, there was ***no evidence of reproductive toxicity*** of 2-ethylhexyl p-methoxycinnamate (EHMC) in Hsd:Sprague Dawley® SD® rats at exposure concentrations of 1000, 3000, or 6000 ppm. Mating and littering were not affected significantly by EHMC exposure.
- Under the conditions of this MOG study, there was ***equivocal evidence of developmental toxicity*** of EHMC in Hsd:Sprague Dawley® SD® rats based on the observed postnatal effects on body weight that showed some indication of recovery by study end, delays in postnatal day 28-adjusted vaginal opening and balanopreputial separation, which could have influenced the apparent transient effects on body weight, and time in estrus was slightly longer in EHMC-exposed females relative to that of the control group. No other signals consistent with alterations in estrogenic, androgenic, or antiandrogenic action were observed. EHMC exposure did not induce any specific fetal malformations.

Questions?