NTP
National Toxicology Program

Draft NTP Technical Report TR597
on
2-Hydroxy-4-methoxybenzophenone
(Feed Studies)

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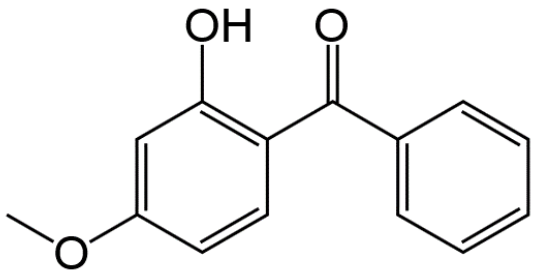
Use and potential exposure to HMB



Ultraviolet filter (Oxybenzone, Benzophenone-3)

- Used in sunscreens and cosmetics (up to 6%)
- Added to plastics to prevent UV-mediated ‘damage’
- Widespread use and exposure
  - Children
  - Higher in women likely as a result of cosmetic use
  - Lifetime
- Public concern

Characterization of potential toxicities to address knowledge gaps

- Hormonal signals (literature)
  - Endocrine Disruptor Screening Panel (EDSP) studies conducted
- Previous NTP study *(Toxicity Report 21)* suggested that the rat testis and ovarian cyclicity are potentially affected
  - Reproduction and developmental outcomes (e.g. viability, growth, terata)
    - Rat NTP Modified One-Generation design
    - NCTR Seg 1 (FEED), 2 (EFD), and 3 (PPND) designs
- FDA Proposed Rule, and sunscreen monograph
  - …*proposed rule is largely focused on potential long-term adverse effects or effects not otherwise readily detected from human use (i.e., carcinogenicity and reproductive toxicity)*
- Bridging dermal to dosed feed oral exposures
  - Pragmatically not possible to conduct littering dermal studies
  - Prefer to group house animals

Chronic/carcinogenicity studies

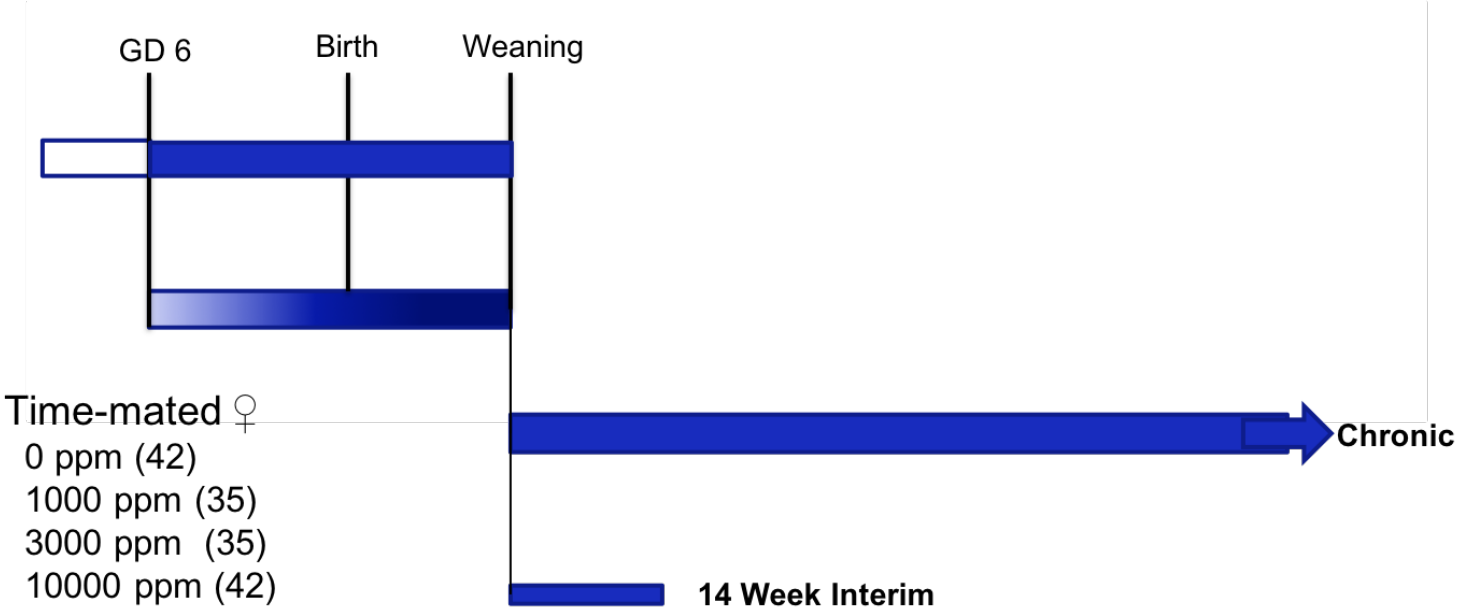
- **Rat including perinatal exposure**
- **Mouse**

Summary of HMB Endocrine Disruptor Screening Results

- "Not Interactive" in the estrogen receptor (ER) binding assay
- Weakly "Positive" in ER transcriptional activation assay; Maximal response ~20% at 100μM; cytotoxicity
- No estrogenic response in the *uterotrophic* assay at 1g/kg
- "Equivocal" in androgen receptor (AR) binding assay; 10^{-4} M did not displace more than 50% of ligand
- "Negative" in AR agonist transcriptional activation assay
- Minimal activity in AR antagonist transcriptional activation assay; >25% at 1.5 mM
- Minimal decreases in testosterone-dependent organ weights at 1g/kg in the Hershberger assay, in the presence of lower body weight

Perinatal Toxicity/Carcinogenicity Study in Hsd:Sprague Dawley SD Rats

- Dose Levels: 0, 1000, 3000, and 10000 ppm in the diet
  Based on 3 month dietary study in F344 rats *(Toxicity Report 21)*
  0, 3125, 6250, 12500, 25000, or 50000 ppm HMB
- Exposure began on gestation day (GD) 6 through lactation; exposure continued after weaning on postnatal day 21 (PND) (50/group)
- 14 week interim necropsy for 0 ppm and 10000 ppm groups (10/sex/group)



NTP Chronic Studies

Perinatal Toxicity/Carcinogenicity Study in Hsd:Sprague Dawley SD Rats

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Standard chronic bioassay in B6C3F1/N Mice

- Dose groups: 0, 1000, 3000, and 10000 ppm in the diet
  Based on previous NTP studies
  - 113, 339, and 1,207 mg/kg for males
  - 109, 320, and 1,278 mg/kg for females

Genetic Toxicity Assessment

Negative in *Salmonella typhimurium* strains TA98, TA100 and *Escherichia coli* strain WP2 *uvr*A pKM101, with and without S9

ADME/TK

- Distribution of HMB following dermal application has been shown to be comparable to that following gavage administration

  Xenobiotica. 2019 Oct 31:1-16. doi: 10.1080/00498254.2019.1680906.

- HMB is detected in rat plasma, highly metabolized and extensively conjugated
- Comparison of the rat data, using internal dose, with human data available in the literature suggests that rat HMB plasma concentrations likely attained in this study are similar to that in humans

  J Anal Toxicol. 2017 Nov 1;41(9):744-754. doi: 10.1093/jat/bkx070.

  JAMA. 2019;321(21):2082-2091. doi:10.1001/jama.2019.5586

Rat
2-Year
Perinatal Phase

Feed and dose consumed during gestation

Week	Feed consumption (g/day)				HMB dose consumed (mg/kg)			
Gest	HMB in diet (ppm)				HMB in diet (ppm)			
	0	1000	3000	10000	0	1000	3000	10000
2	17.9	18.2	17.7	15.9	-	71	211	644
3	20.3	20.2	19.5	18.5	-	63	189	616

Feed and dose consumed during gestation and lactation

Week	Feed consumption (g/day)				HMB dose consumed (mg/kg)			
Gest	HMB in diet (ppm)				HMB in diet (ppm)			
Lac	0	1000	3000	10000	0	1000	3000	10000
2	17.9	18.2	17.7	15.9	-	71	211	644
3	20.3	20.2	19.5	18.5	-	63	189	616
1	38.2	38.0	37.4	39.4	-	133	403	1436
2	54.4	54.1	53.4	52.3	-	173	522	1719
3	66.1	66.5	67.3	65.5	-	212	684	2180

Post weaning F_1 HMB consumption for the 1000, 3000, and 10000 ppm groups:

- 58, 168, and 585 mg/kg/day for the males
- 60, 180, and 632 mg/kg/day for the females

No HMB-related effects on food consumption

Reproductive Parameters

No effects on:

- Pregnancy
- Litter size, sex ratio
- PND 1 male or female pup weights
- Pup viability

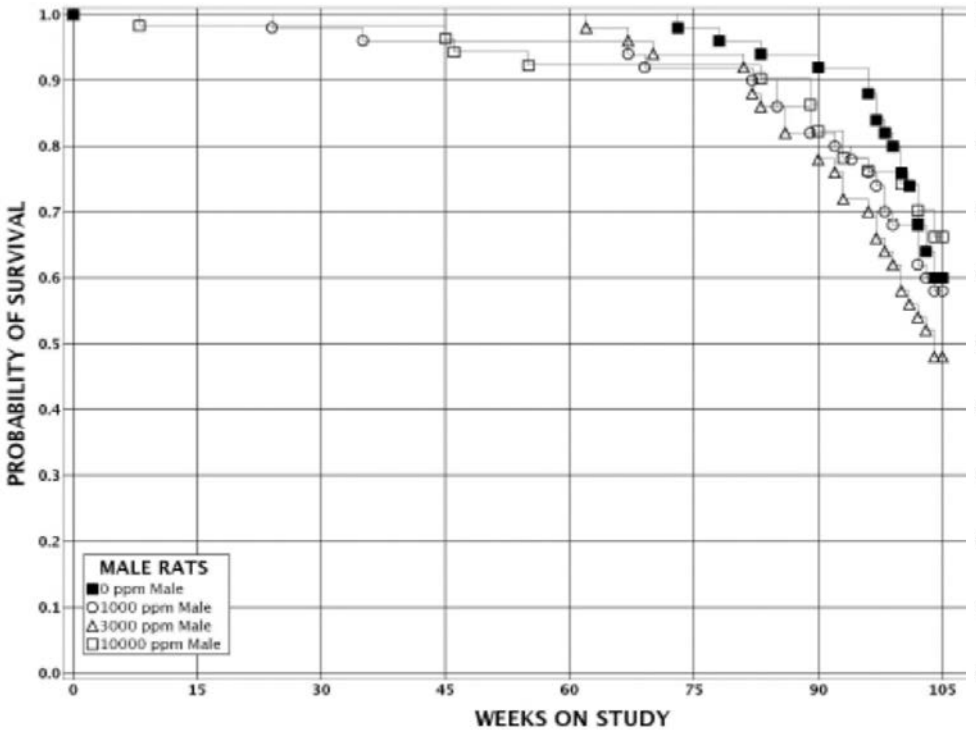
Pup body weights in the 10000 ppm group were subsequently lower (~10%)

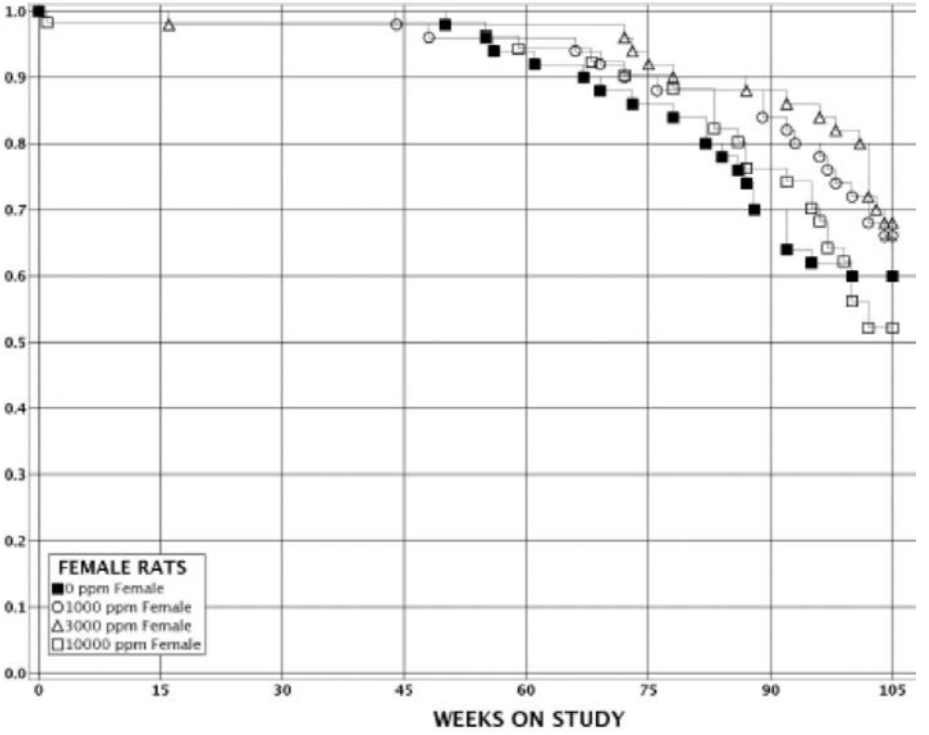
Rats

Chronic Phase

2-year terminal

Survival

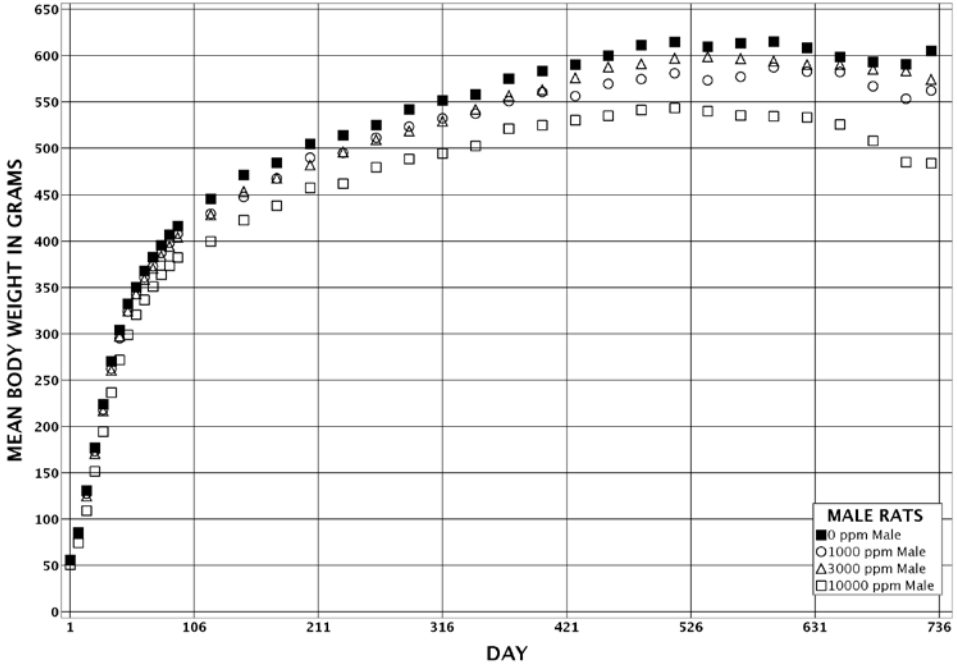


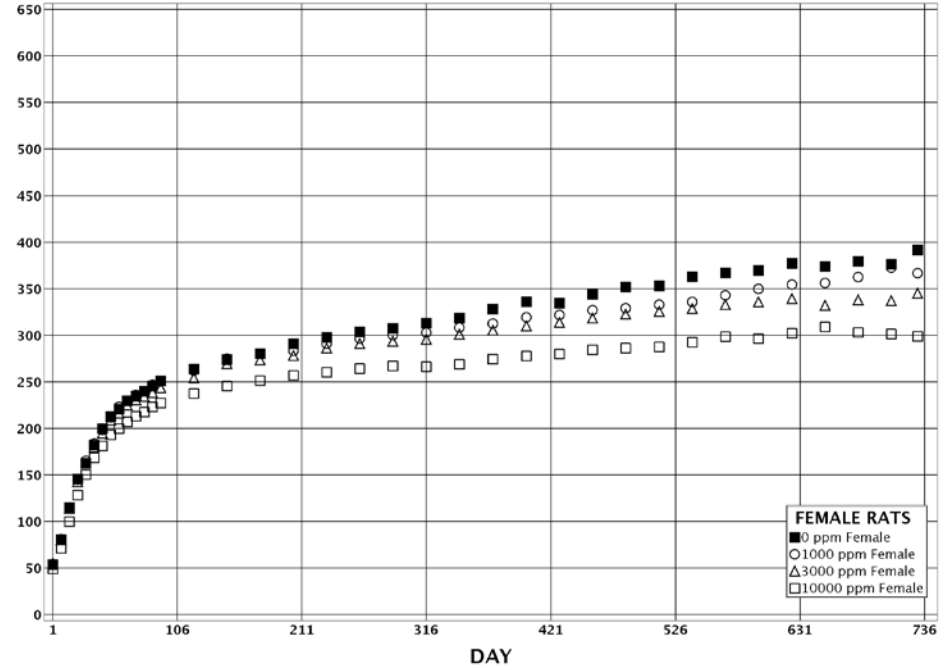


No HMB-related effects on survival

Body Weights

	0 ppm	1000 pm	3000 ppm	10000 ppm
Males				
Body Weight (g)	605.1	562.4	574.2	484.1
Body Weight (%)	-	92.9%	94.9%	80.0%
Females				
Body Weight (g)	391.8	366.8	345.3	299.3
Body Weight (%)	-	93.6%	88.2%	76.4%





Males: Malignant Meningioma

Males	Historical Control	0 ppm	1000 ppm	3000 ppm	10000 ppm
BRAIN		50	50	50	50
Meningioma Malignant	0/340	0	1	3	0
BRAIN and SPINAL CORD					
Meningioma Malignant	0/340	0	1	4	0

Males: Malignant Meningioma

Males	Historical Control	0 ppm	1000 ppm	3000 ppm	10000 ppm
BRAIN		50	50	50	50
Meningioma Malignant	0/340	0	1	3	0
BRAIN and SPINAL CORD					
Meningioma Malignant	0/340	0	1	4	0

Equivocal evidence of carcinogenic activity based on the occurrence of malignant meningiomas of the brain and spinal cord

Female: Thyroid Gland

Females	Historical Control	0 ppm	1000 ppm	3000 ppm	10000 ppm
THYROID GLAND, C-CELL		50	50	50	50
Adenoma	38/339 (4-22%)	5	11	17*	10
Carcinoma	4/339 (0-4%)	1	1	0	1
Hyperplasia, Focal		11	11	9	9

No apparent progression to carcinoma; no increase in the incidence of hyperplasia

* $p \leq 0.05$

Female: Thyroid Gland

Females	Historical Control	0 ppm	1000 ppm	3000 ppm	10000 ppm
THYROID GLAND, C-CELL		50	50	50	50
Adenoma	38/339 (4-22%)	5	11	17*	10
Carcinoma	4/339 (0-4%)	1	1	0	1
Hyperplasia, Focal		11	11	9	9

No apparent progression to carcinoma; no increase in the incidence of hyperplasia

Equivocal evidence of carcinogenic activity based on the higher incidence of C-cell adenomas of the thyroid gland

* $p \leq 0.05$

Female: Uterine Findings

Females	Historical Control	0 ppm	1000 ppm	3000 ppm	10000 ppm
UTERUS		50	50	50	50
Polyp Stromal	34/150 (16-32%)	8	15	18*	10
Stromal Sarcoma	3/150 (0-4%)	0	1	2	0
Stromal Sarcoma or Polyp Stromal	36/150 (16-32%)	8	15	19*	10

No apparent increase in the incidence of endometrial adenocarcinoma

* $p \leq 0.05$

Female: Uterine Findings

Females	Historical Control	0 ppm	1000 ppm	3000 ppm	10000 ppm
UTERUS		50	50	50	50
Polyp Stromal	34/150 (16-32%)	8	15	18*	10
Stromal Sarcoma	3/150 (0-4%)	0	1	2	0
Stromal Sarcoma or Polyp Stromal	36/150 (16-32%)	8	15	19*	10

No apparent increase in the incidence of endometrial adenocarcinoma

Equivocal evidence of carcinogenic activity based on the higher incidence of stromal polyp of the uterus

* $p \leq 0.05$

Female: Reproductive Tract

Females	Historical Control	0 ppm	1000 ppm	3000 ppm	10000 ppm
Uterus		50	50	50	50
Endometrium, Atypical Hyperplasia		9	14	19*	14
Adenocarcinoma	11/150 (2-10%)	5	3	0*	4
Endometrium, Metaplasia, Squamous		36	35	25*	32

Increased incidences of atypical hyperplasia of the endometrium

* $p \leq 0.05$

Female: Adrenal Cortex

Females	0 ppm	1000 ppm	3000 ppm	10000 ppm
ADRENAL CORTEX	50	50	50	50
Hypertrophy, Focal (includes bilateral)	*25*	42**	39*	27

Increased incidences of focal hypertrophy of the adrenal cortex

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

Male: Testes

Males	0 ppm	1000 ppm	3000 ppm	10000 ppm
TESTES	50	50	50	50
Arteriole, Necrosis, Fibrinoid	16*	19	16	25*
Interstitial Cell, Hyperplasia	1*	0	0	5

Increased incidence of fibrinoid necrosis of the arterioles and interstitial cell hyperplasia

* p≤0.05

Mice

2-Year

Dose Selection Rationale

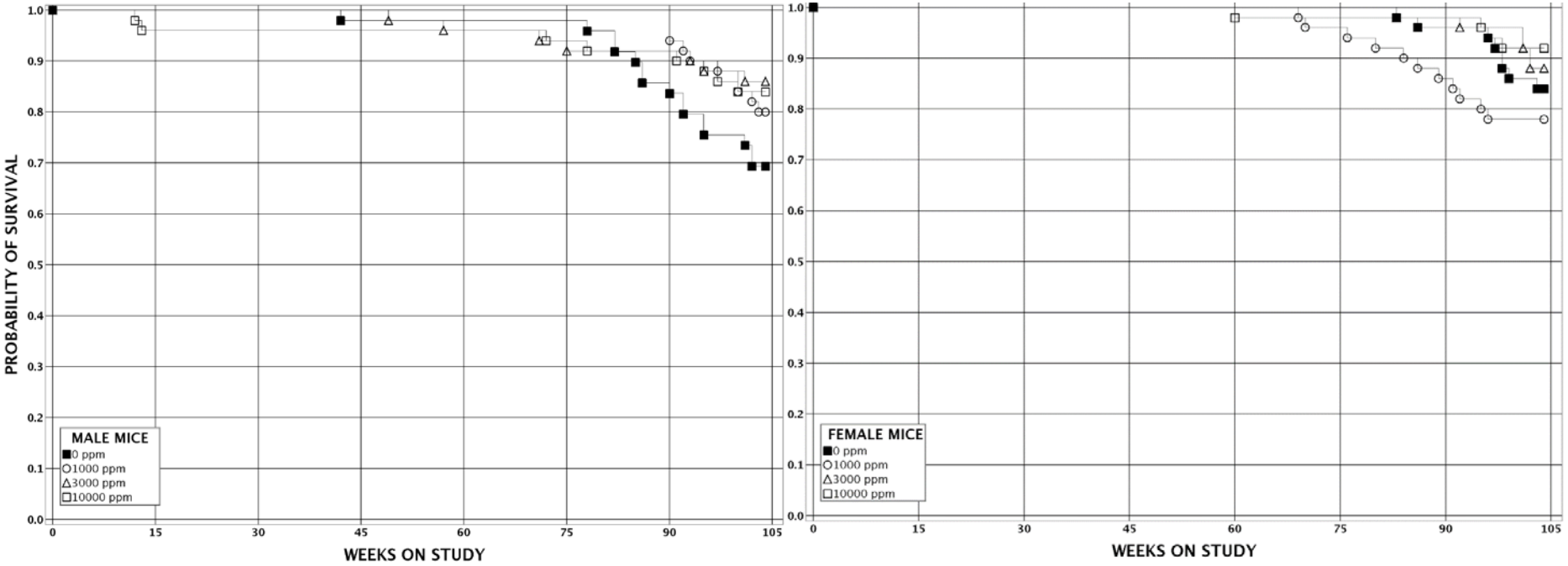
- 13 Week NTP study *(Toxicity Report 21)*

  0, 3125, 6250, 12500, 25000, 50000 ppm

- Lower body weights at ≥12500 ppm
- Kidney and liver lesions
  - Increase in liver weights at ≥6250 ppm (males) and at ≥3125 ppm (females)
  - Increase in kidney weight and histopathology at >25000 ppm
  - Elevated liver enzymes; 25000 and 50000 ppm (females)

0, 1000, 3000 and 10000 ppm were selected (same as the rat study)

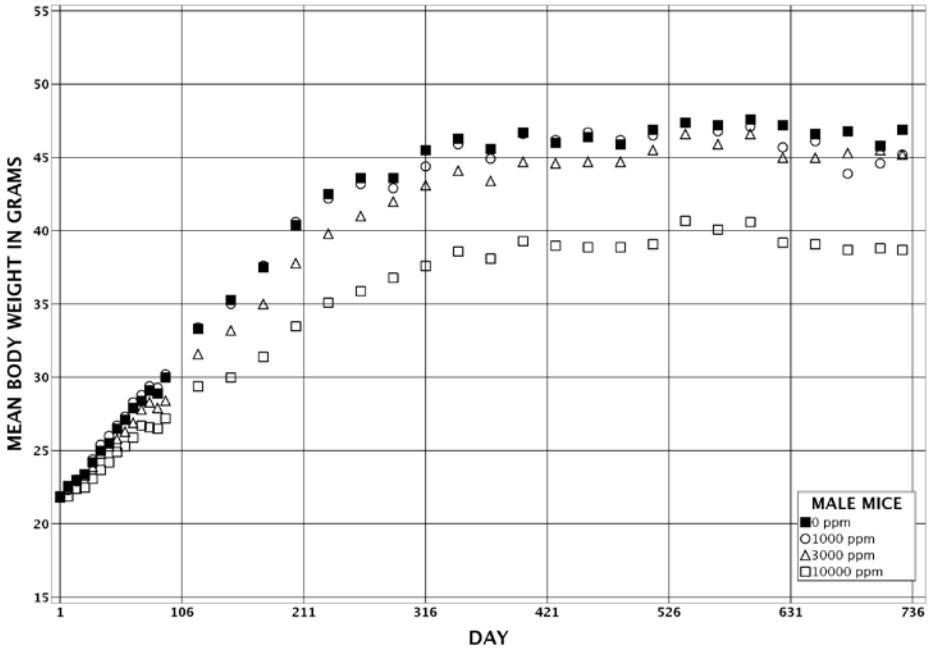
Survival

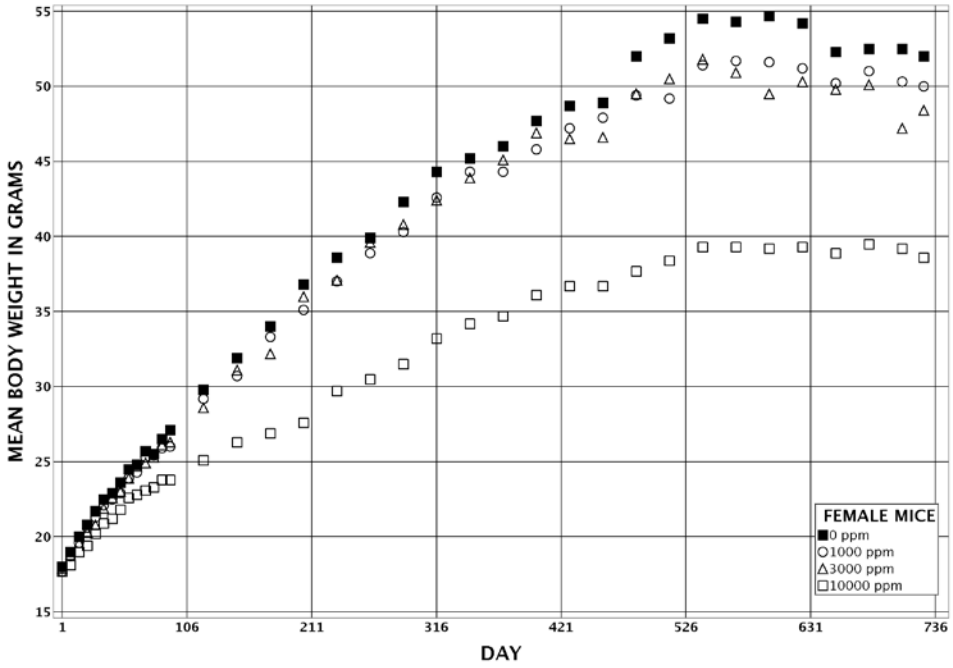


No HMB-related effects on survival

Body Weight

Males	0 ppm	1000 pm	3000 ppm	10000 ppm
Body Weight (g)	46.9	45.2	45.2	38.7
Body Weight (%)	-	96.3%	96.4%	82.6%
Females				
Body Weight (g)	52	50	48.4	38.6
Body Weight (%)	-	96.2%	93.1%	74.4%





There was *no evidence of carcinogenic activity* in male or female B6C3F1/N mice at exposure levels of 1000, 3000, and 10000 ppm.

Male: Nonneoplastic Lesions

Males	0 ppm	1000 ppm	3000 ppm	10000 ppm
Bone marrow: pigment	3**	2	9	50**
Spleen: pigment	4**	5	10	17**
Liver: hepatocyte, syncytial alteration	2**	39**	45**	48**
Kidney: chronic progressive nephropathy	41*	48	48*	50*
renal tubule, cytoplasmic alteration	0**	0	0	46**
infiltration cellular, lymphocytes	40*	40	43	46*

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

Female: Nonneoplastic Lesions

Females	0 ppm	1000 ppm	3000 ppm	10000 ppm
Bone marrow: pigment	6**	0*	0*	50**
Spleen: pigment	12**	10	36**	38**
Kidney: osseous metaplasia	0*	1	3	5*

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

Conclusions

Male Hsd:Sprague Dawley SD rats

- *Equivocal evidence of carcinogenic activity*
  - Occurrence of brain and spinal cord malignant meningiomas

- Exposure to 2-hydroxy-4-methoxybenzophenone resulted in increased incidences of nonneoplastic lesions of the testis and pancreas in male rats.

Female Hsd:Sprague Dawley SD rats

- *Equivocal evidence of carcinogenic activity*
  - Increased incidence of thyroid C-cell adenomas
  - Increased incidence of uterine stromal polyps

- Exposure to 2-hydroxy-4-methoxybenzophenone resulted in increased incidences of nonneoplastic lesions of the uterus and adrenal cortex in female rats.

Conclusions

Male and Female B6C3F1/N mice

- *No evidence of carcinogenic activity* at 1,000, 3,000, and 10,000 ppm of 2-hydroxy-4-methoxybenzophenone

- Exposure to 2-hydroxy-4-methoxybenzophenone resulted in increased incidences of nonneoplastic lesions of the bone marrow, spleen, and kidney in male and female mice, and liver in male mice.

Questions?