Assessing the reproductive and developmental toxicity of parabens

Presentation to CIR

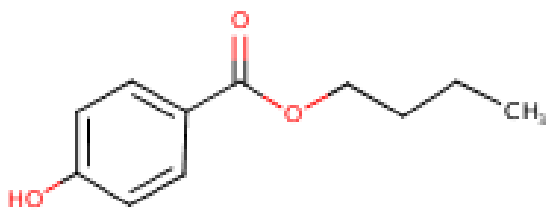
March 5, 2018

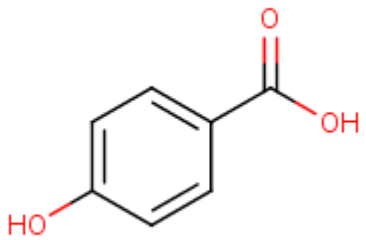
Overview

- Mode of action
- Metabolism
- Toxicity and risk

Mode of Action

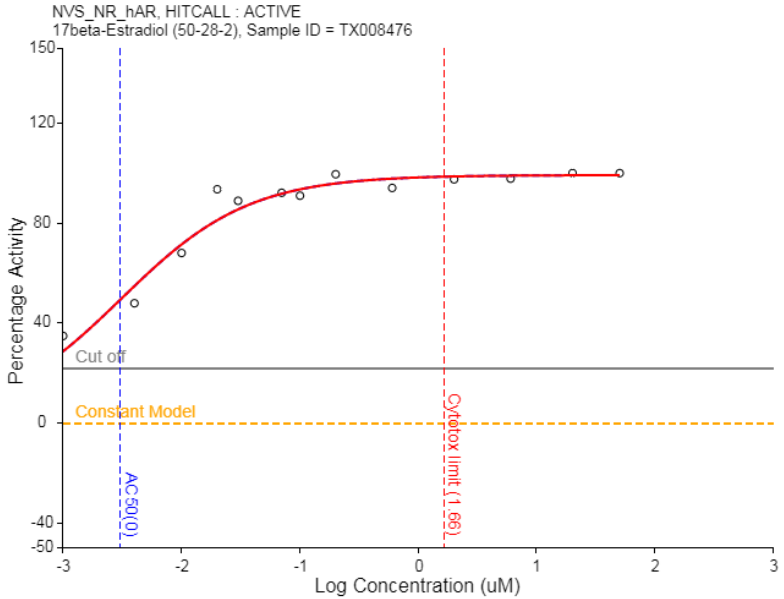
- Weak estrogen receptor agonist
  - In vitro displacement of estradiol from both isoforms of ER, transcription of E2-responsive genes
  - Butylparaben is 10,000-100,000x less potent than estradiol, methylparaben 1,000,000, and propyl and ethyl are in between
  - No activity for p-hydroxybenzoic acid

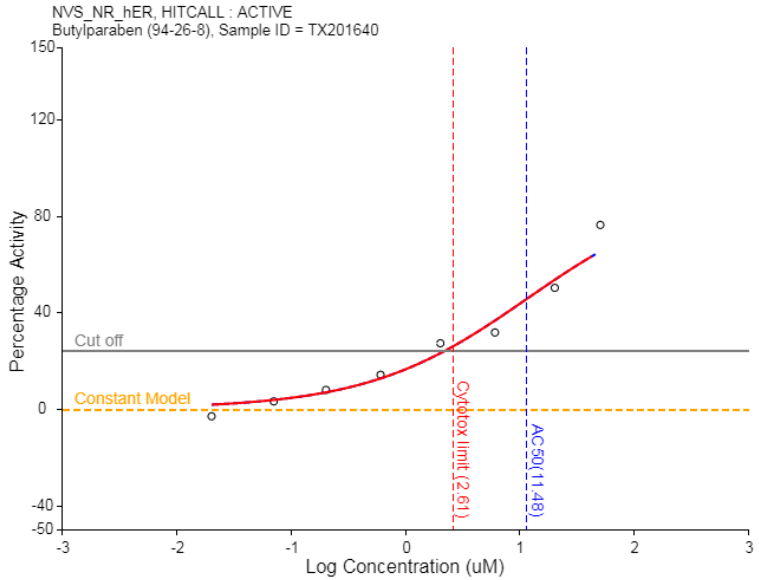




EDSP21 results

Chemical	# of positive estrogenicity results (out of 18)
17-beta-estradiol	17
Butyl paraben	15
Propyl paraben	14
Ethyl paraben	11
Methyl paraben	5
p-hydroxybenzoic acid	2

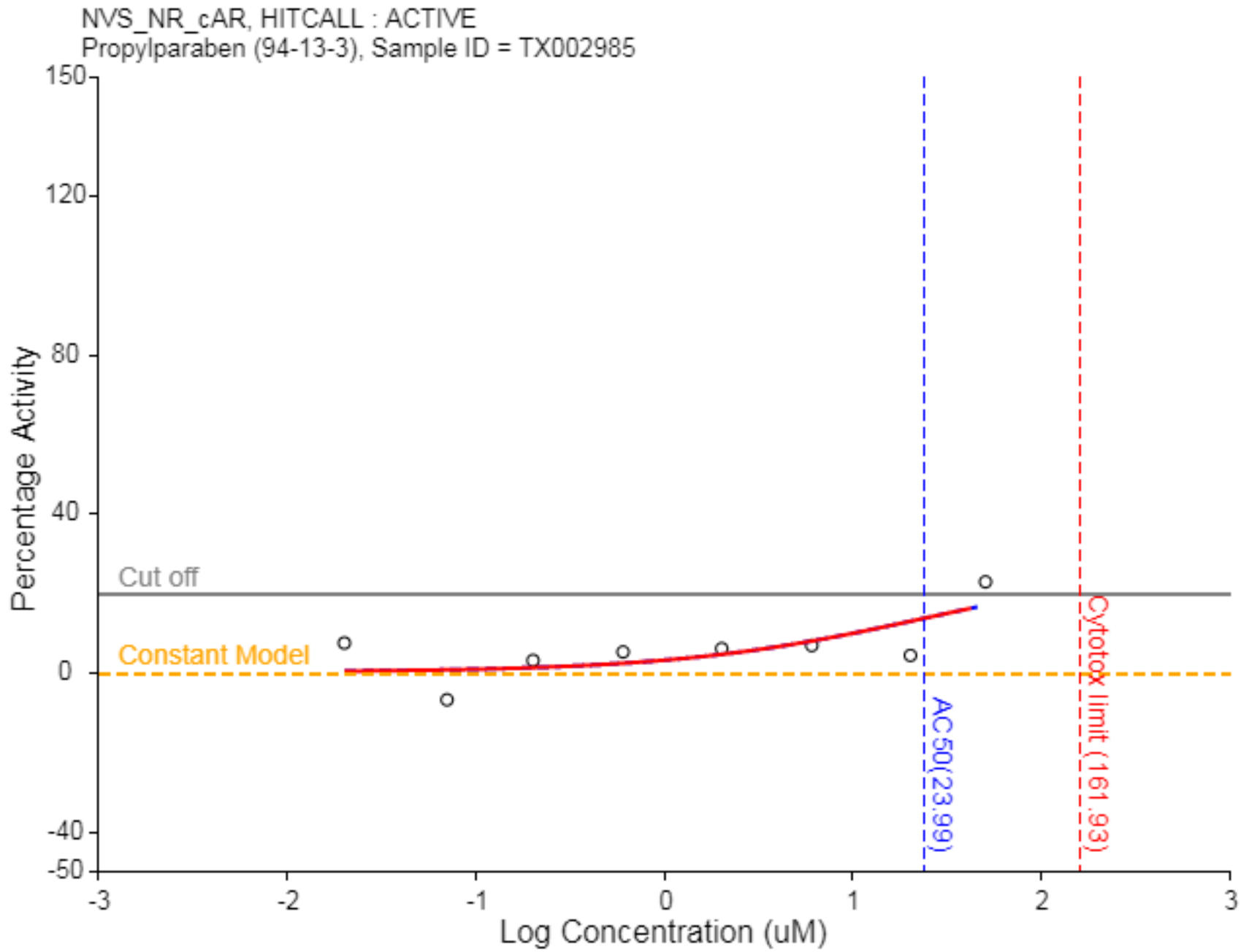




Are parabens anti-androgens?

- Positive results in one or two in vitro reporter gene assays
- EDSP21 results

Chemical	# of positives
Butylparaben	0/10
Propylparaben	1/9
Ethylparaben	0/11
Methylparaben	0/8


NVS_NR_cAR, HITCALL : ACTIVE
Propylparaben (94-13-3), Sample ID = TX002985
150
120
80
40
0
-40
-50
Percentage Activity
Cut off
Constant Model
AC50(23.99)
Cytotox limit (161.93)
-3
-2
-1
0
1
2
3
Log Concentration (uM)

Effects of an estrogen agonist in vivo on male rat offspring

- Decreased body weight gain (probably as a result of decreased T)
- Decreased epididymis weight
- Decreased circulating T and LH (no effect on FSH)
- Decreased epididymal sperm number
- Slight decrease in normal sperm morphology (97% vs 99%)
- No effect on sperm motility

- From Cook et al. (1998) Tox. Sci. 44: 155-168, a one-generation study with 17-beta-estradiol

Effects of anti-androgens in vivo on male rat offspring development

- Malformations of the reproductive tract
  - Undescended testes
  - Urethral malformations, including hypospadias
  - Small prostate, seminal vesicles
- Marked decreases in anogenital distance (40-60% in male pups early, 10% in adult offspring)
- Later puberty
- Decreased serum T
- Decreased epididymal sperm concentration
- Areola/nipple retention

Metabolism

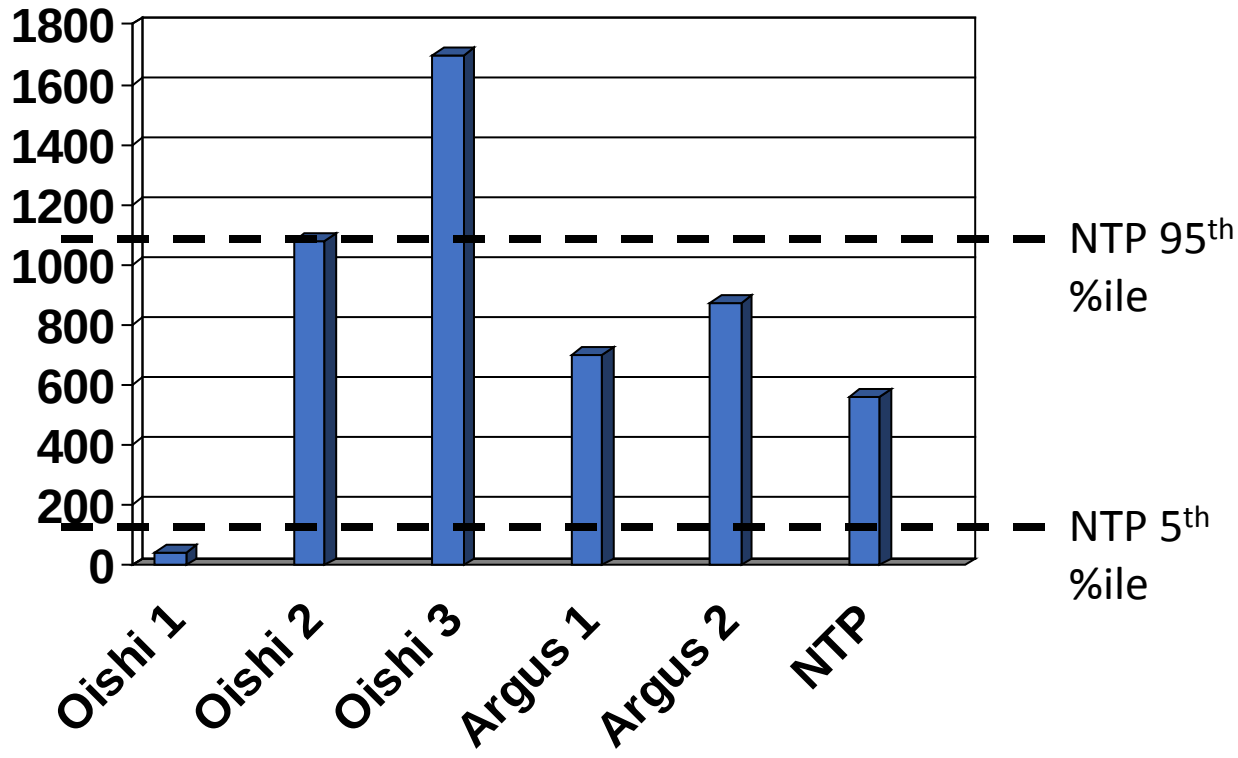
- Parabens are rapidly hydrolyzed at portals of entry (dermal and oral)
- Products are p-hydroxybenzoic acid and short-chain alcohols
- Clinical studies measuring absorption show only very small percentage of paraben in plasma (approx. 2% of administered dose)

- Therefore, studies using sc injection might be useful for understanding the hazard, but not the risk of parabens

Biological effects and toxicity

- Estrogenicity in vivo?
  - Uterotrophic studies show a lack of effect from oral exposures (up to 1200 mg/kg/day BP), a blunted effect from sc exposures (400-1200 mg/kg/day BP)
- Toxicity
  - No developmental toxicity in a guideline study up to 1000 mg/kg/day by gavage
  - Some reports of effects on male reproductive development when exposure was early postnatal (Oishi)
  - Failure to replicate in a GLP study (Hoberman et al.)
  - Oishi's results were inconsistent with historical data for the affected parameters

Control Sperm Concentration



CIR Questions: 1

- Is epididymal sperm concentration a relevant DART endpoint for defining a NOAEL?
- Yes. Epididymal sperm concentration is highly correlated with sperm count, and a decrease in sperm count would increase the risk of infertility. Like any individual measurement, epididymal sperm concentration should be viewed in the context of the weight of evidence. A lack of effect on testicular or epididymal histology would tend to decrease the validity of the effect.

- NB: The NOAEL for sc injection would not be a relevant point of departure for risk assessment because it circumvents portal of entry metabolism

CIR Questions: 2

- Is anogenital distance a relevant DART endpoint on which to base a NOAEL?
- No. AGD on its own should be considered to be a biomarker of effect and not an adverse outcome.

Recent animal studies

- Garcia et al (2017): butylparaben, sc exposure, young male rats
- Taxvig et al (2008): butylparaben or ethylparaben, sc, gestational
- Zhang et al (2014): butylparaben, oral, gestational and early postnatal
- Boberg et al (2016): butylparaben, oral, gestational and early postnatal

- Manservisi et al (2015): a very low dose of methylparaben, two-gen study: high rate of pup mortality in every group, not consistent with any other study
- Gazin et al (2013): propylparaben to juvenile male rats, no effects on repro parameters up to 1000 mg/kg/day oral

Garcia et al (2017)

- 6 week old male rats
- Sc injection, 3 x per week, for 57 days
- Two control groups: vehicle and untreated
  - Statistical comparisons appear to have been done vs the untreated control, even though there were big differences between this and the vehicle control

Garcia et al (2017)

Parameter	Untreated	Control	150 mg/kg	300 mg/kg	600 mg/kg
Prostate weight (g/kg bw)	1.53 (0.32)	1.90 (0.24)	1.98 (0.26)*	1.86 (0.27)	2.25 (0.24)
Epididymal sperm conc. (million/ml)	400 (26)	300 (146)	174 (85)*	149 (56)*	205 (56)*
Testicular spermatid conc. (million/ml)	21.5 (36.2)	15.2 (10.6)	14.7 (16.1)	21.0 (79.3)	13.4 (15.1)*
% progressively motile sperm	60 (8)	52 (9)	48 (7)*	46 (8)*	47 (7)*
% normal sperm	75 (7)	72 (5)	67 (6)*	55 (5)*	50 (6)*

Charles River historical control ranges:
Motility: 57-80%
Normal sperm: 86-98%

Taxvig et al (2008)

- Ethylparaben (400 mg/kg/day) or butylparaben (200 or 400 mg/kg/day), sc, GD 7-21
- No effects on AGD or other parameters in fetuses, including sex steroid levels
- Effects on some adrenal steroid synthesis gene expression in females but not males

Boberg et al (2016)

- Butylparaben, oral gavage, GD7 – PND 22
- 13-17 litters per group
- 10, 100, 500 mg/kg/day
- Effects on AGD, male and female, two higher dose levels (around 10%, not obviously dose-related)
- No effect on areola/ nipple retention
- Decreased ventral prostate weight and seminal vesicle weight on PND 80-90 in high dose group (vs. increased prostate weight in Garcia et al)
- Effects on prostate and mammary gland histology at higher dose levels, not dose-responsive

Zhang et al (2014)

- Butylparaben, oral, GD 7- PND 21, 64, 160, 400, 1000 mg/kg/day
- Only 7-8 litters per group
- AGD decreased in males at two higher dose levels, PND 1 and 21 (approx. 10%) but data not normalized to body mass
- 3-4 day delay in preputial separation at two high higher dose levels, but body weight at PPS was the same across groups
- Decreased serum T and LH over different ages at high dose
- Decreased epididymal sperm concentration and testis spermatid concentration at two higher dose levels
  - However, all values, including control appear to be far below historical control range
- Reported effects on histology at two higher dose levels, but suboptimal tissue preparation

Human studies

- Meeker et al (2011)
- Adoamnei et al (2018)
- Nassan et al (2017)
- Jurewicz et al (2017)

Meeker et al 2011

- Semen collection in patients at an infertility clinic and urinary measurements of various parabens and bisphenol A
- No relationship between any chemicals and semen or hormone parameters
- No interquartile effects (e.g., lowest quartile vs highest quartile) in sperm DNA damage for butylparaben but a significant trend test ($p=0.03$) across quartiles
- Authors make no definitive conclusions

Adoamnei et al (2018)

- Controlled study in university students (presumed fertile)
- Measurement of serum hormones, semen parameters, and urinary paraben levels
- No association between paraben levels and any measured parameter

Jurewicz et al (2017)

- 315 men visiting an infertility clinic, sperm conc. 15-300 million/ml
- Semen parameters, sperm DNA stability, serum hormones
- Urinary paraben measurement
- Frequency of detection

MP	EP	PP	BP	iBP
99	42	89	11	16

Jurewicz et al (2017)

- When paraben was below LOD, a value of LOD/2 was used
- As a consequence, the only statistics for EP, BP and isobutylP were for a group where the authors acknowledge that >75% of samples had no detectable paraben
- Significant p-value for
  - sperm morphology for EP and BP, but not PP
  - serum T for BP
  - High DNA stability for isobutylP
- 121 statistical comparisons, 4 had p values of $p<0.05$

Nassan et al (2017)

- Biomonitoring study
- Ability to detect urinary metabolites of parabens and monoethyl phthalate 6 hours after use of personal care products

CIR Questions: 3

- Are there reasons to elevate or discount any of the DART data?
  - Studies using sc injection are of interest as support, but not appropriate for risk assessment
  - Mode of action data are important in weighing consistency of data
    - Strong evidence that some parabens are weakly estrogenic
    - Preponderance of evidence that parabens are not anti-androgens
    - Taxvig et al suggest an effect on steroid synthesis, but results are not strong
  - Effects of estradiol:
    - Decreased body weight gain (probably because of decreased T)
    - Decreased epididymis weight
    - Decreased epididymal sperm concentration
    - Decreased T and LH
    - Slight effect on sperm morphology

Summary of recent animal studies

- Effects on AGD
  - Yes for Boberg and Zhang (but only 10%, not dose-responsive)
  - No for Taxvig
- Effect on serum T, LH
  - Yes for Zhang at 1000 mg/kg/day
- Effects on epididymal sperm concentration
  - Yes for Boberg (10, 100 and 500 mg/kg/day, no dose-response)
  - Yes for Zhang (400 and 1000 mkd, NOAEL = 160 mkd) (data outside HCD)
  - No for Hoberman up to 1000 mkd (but different dosing period)
- Effects on testicular spermatid concentration
  - Yes for Zhang (400 and 1000 mkd, NOAEL = 160)
  - No for Hoberman

CIR Questions: 4

- What is an appropriate DART NOAEL to use to calculate MOS?

Dosage (mkd)	10	64	100	160	400	500	1000
AGD	-	-	+	-	+	+	+
Serum T, LH		-		-	-		+
Epid. sperm conc.	+	-	+	-	+	+	+
Testis sperm conc.		-		-	+		+
Histology	-	-	+	-	+	+	+

Red = not dose-responsive

Conclusions

- Lots of conflicting data
- Mode of action studies: weak in vitro estrogens
- Metabolism: high level of hydrolysis at portals of entry
  - Explains lack of an in vivo estrogenic effect (uterotrophic assay) by oral or dermal route
- Two oral studies (Boberg, Zhang) report effects using a prenatal/perinatal dosing paradigm not used by others, some of which are consistent with an estrogen mechanism
  - Is there a downregulation of esterase activity during pregnancy/lactation in the rat?
- 400 mkd is a pragmatic LOAEL, 160 mkd NOAEL, from which to calculate MOS for butylparaben. Assuming an estrogenic mechanism, this would be adequately protective for propyl, ethyl and methylparaben