Animal Studies of Reproductive and Developmental Toxicity of Chloroform

Animal Studies of the Effects of Chloroform on DART Endpoints

1. Developmental Toxicity

a) Rats, inhalation (5) and oral (3) routes
b) Mice, inhalation (1) and oral (1) routes
c) Rabbits, oral (1) route only
d) Zebrafish, *in vitro* (1)

2. Female Reproductive Toxicity

a) Rats, inhalation (3) and oral (2) routes
b) Mice, inhalation (1) and oral (2) routes
c) Rabbits, oral (1) route only
d) Beagle dogs, oral (1) route only

3. Male Reproductive Toxicity

a) Rats, oral (1) route only
b) Mice, inhalation (1) and oral (1) routes
c) Beagle dogs, oral (1) route only

4. Multi-generation Reproductive Toxicity Study With Satellites

a) Mouse, oral (1) route
b) Data relevant to developmental, female, and male reproductive toxicity

Developmental Toxicity of Chloroform in the Rat, Inhalation Route

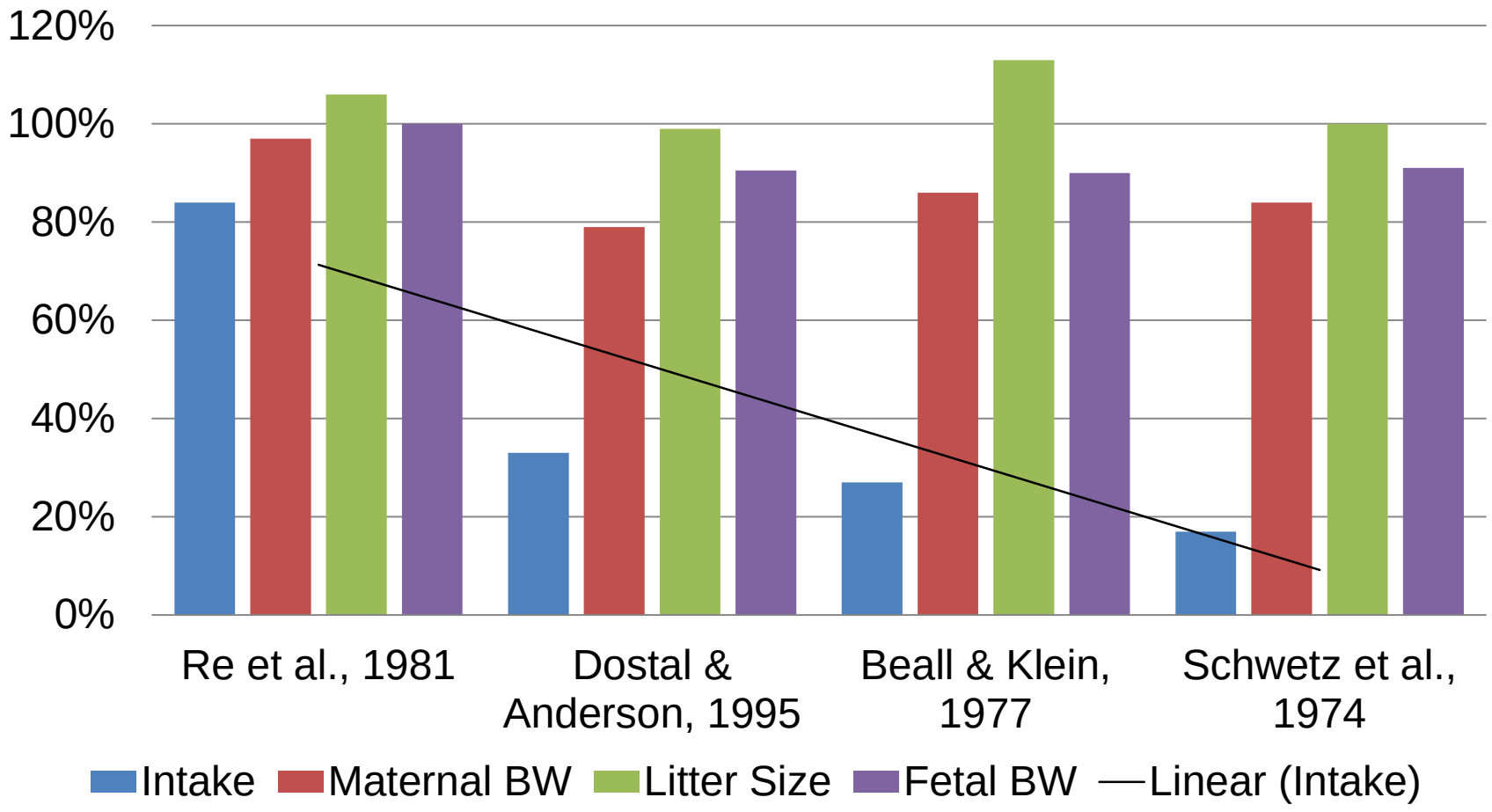
Reference	Maternal Effects	Developmental Effects
Schwetz et al., 1974	30 ppm: ↓ BW* on GD-13 (~90% of control)	30 ppm: ↑ skeletal anomalies & ↓ CRL^
Baeder & Hoffman, 1988	30 ppm: ↓ BW on GD-17 (~92% of control)	30 ppm: ↑ totally resorbed litters (early implantation), ↓ CRL
Baeder & Hoffman, 1991	10 ppm: ↓ BW on GD-21 (~95% of control)	3, 10, 30 ppm ↑ ossification variations/fetus 30 ppm: ↓ fetal wt & CRL

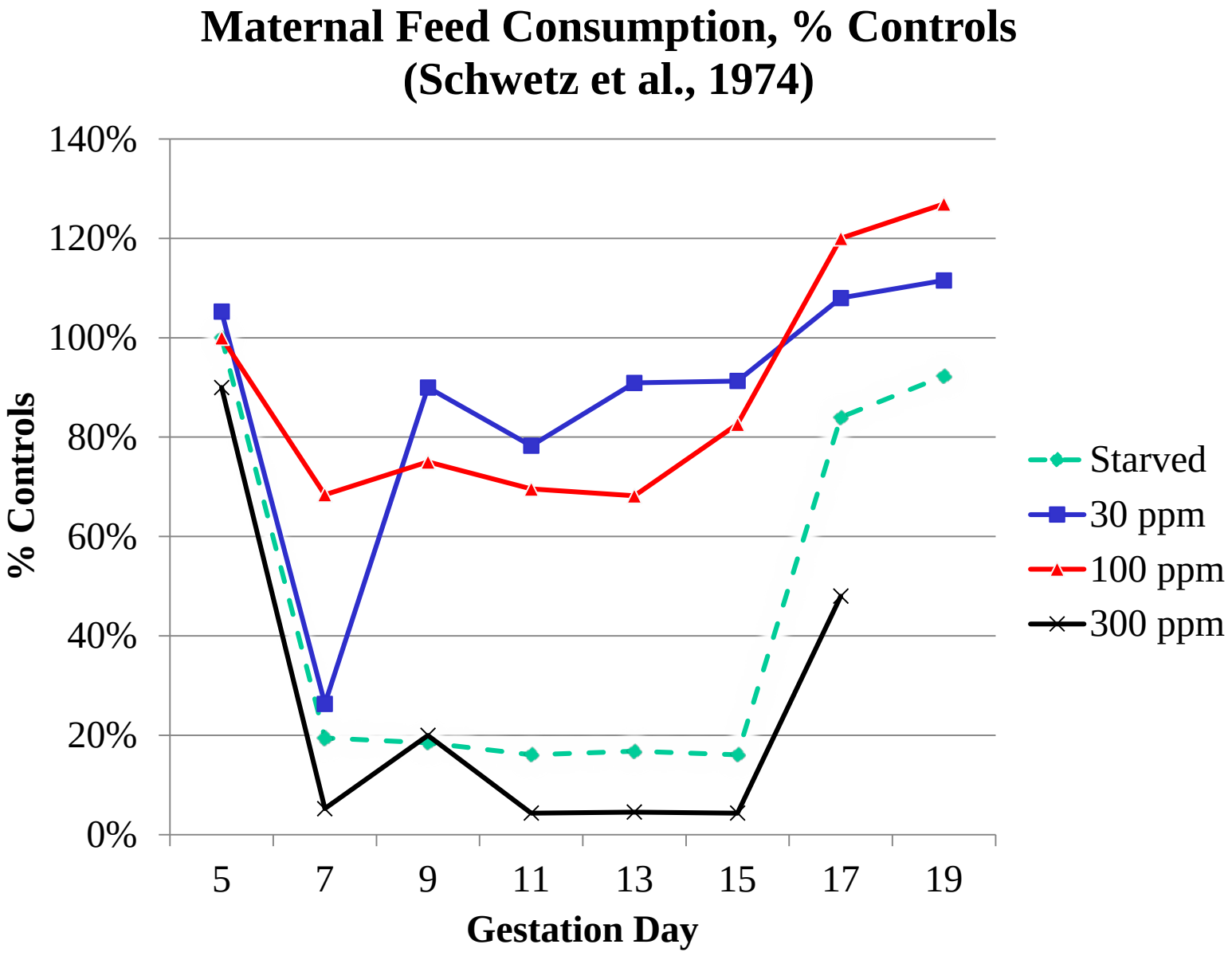
*BW = body weight
^CRL = crown-rump length

Interpreting Developmental Toxicity With Concurrent Maternal Toxicity

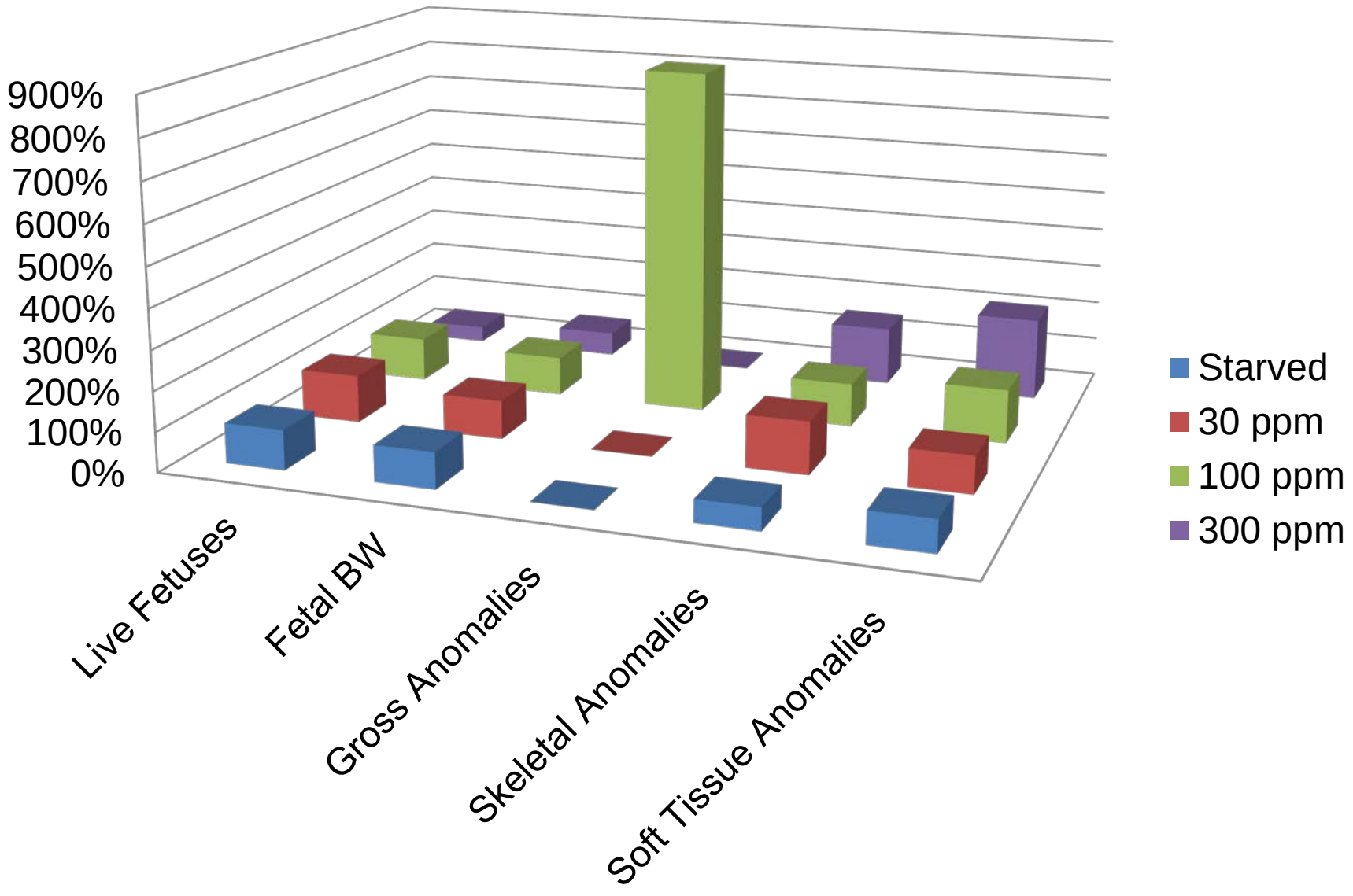
- **US EPA risk assessment guidelines**:
  - Standard teratology studies are designed to produce *minimal* maternal toxicity at the highest dose tested
  - Effective doses for dam and offspring may simply overlap
  - Agents having minimal adult toxicity may have permanent effects on developing offspring
  - *Excessive* maternal toxicity (e.g. mortality $\geq$ 10%) may obscure interpretation of developmental data
- **Data on maternal feed restriction and development**:
  - Species of test animal, sensitivity: mice > rats > rabbits
  - Duration and timing of restriction
  - Severity of restriction

Developmental Effects of Maternal Feed Restriction, Sprague Dawley Rats

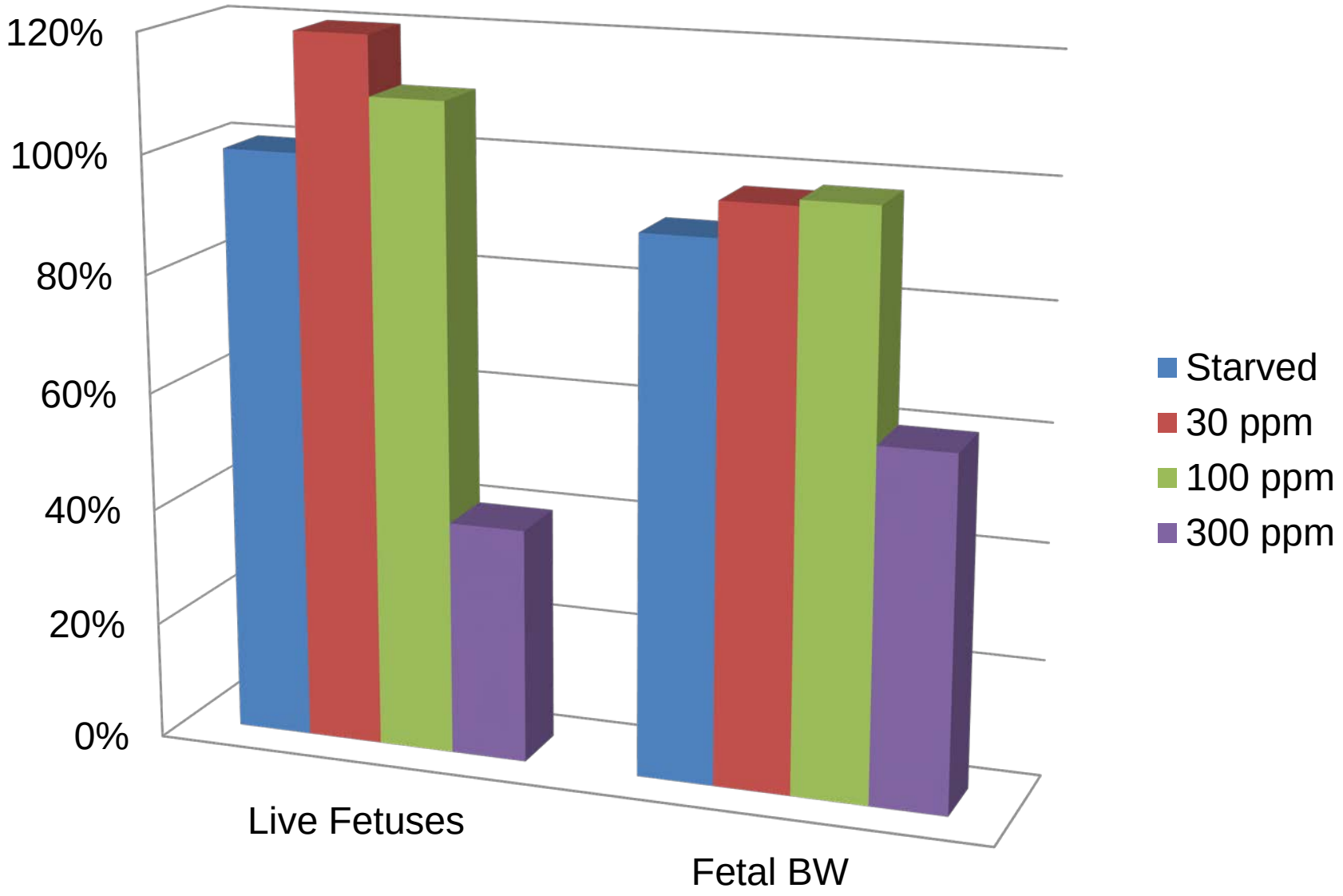



Maternal Feed Consumption, % Controls
(Schwetz et al., 1974)
140%
120%
100%
80%
60%
40%
20%
0%
% Controls
5
7
9
11
13
15
17
19
Gestation Day
Starved
30 ppm
100 ppm
300 ppm

Fetal Data, % Controls (Schwetz et al, 1974)



Fetal Data, % Controls (Schwetz et al, 1974)



Developmental Toxicity of Chloroform in the Mouse, Inhalation Route

Reference	Maternal Effects	Developmental Effects
Murray et al., 1979	GD 1-7: ↓ wt gain GD 6-15: 1/35 maternal death ↑ liver wt GD 8-15: ↓ wt gain ↑ liver wt	GD 1-7: ↑resorptions (2 litters totally resorbed) ↓pregnancy rate, fetal BW & CRL ↑delayed ossification of sternebrae and skull bones GD 6-15: ↓pregnancy rate ↑delayed ossification of skull bones GD 8-15: ↓fetal BW & CRL ↑delayed ossification of sternebrae and skull bones ↑cleft palate

Developmental Toxicity of Chloroform in the Rat, Oral Route

Reference	Maternal Effects	Developmental Effects
Thompson et al, 1974 (range-finding study)	316 mg/kg-day: ↓ wt gain (no details) 1/6 maternal death	316 mg/kg-day: ↑ resorptions ↓ litter size and fetal wt
Thompson et al, 1974 (full Teratology study)	126 mg/kg-day: ↓ wt gain (no details)	126 mg/kg-day: ↓fetal wt ↑ fetuses with bilateral extra lumbar ribs
Ruddick et al., 1983	400 mg/kg-day: ↓ wt gain ↑ relative liver & kidney wts Altered blood biochem	400 mg/kg-day: ↓ fetal wt ↑ aberrant sternebrae ↑ runts

Developmental Toxicity of Chloroform in the Mouse, Oral Route

Reference	Maternal Effects	Developmental Effects
Burkhalter & Balster, 1979; Balster & Borzelleca, 1982	Not discussed	31.1 mg/kg-day observed at birth: No effect on live litter size

Developmental Toxicity of Chloroform in the Rabbit, Oral Route

Reference	Maternal Effects	Developmental Effects
Thompson et al, 1974 (range-finding study)	63 mg/kg-day: wt loss, 1/5 death	63 mg/kg-day: ↑ abortion of litters ↓ fetal viability in litters with live fetuses
Thompson et al, 1974 (full Teratology study)	20 mg/kg-day: Clinical symptoms during treatment	20 mg/kg-day: Aborted litters (also seen in controls and higher doses; no clear dose effect) ↓ fetal wt ↑ fetal incidence of incompletely ossified skull bones

Developmental Toxicity of Chloroform in Zebrafish, *in vitro*

Reference	Developmental Effects
Teixidó et al., 2015	**EC_{20}** = 0.7 mM (84.7 mg/L) **EC_{50}** = 0.85 mM (100.3 mg/L) **LC_{50}** = 2.1 mM (286.5 mg/L) **TI*** (LC_{50}/EC_{50}) = 2.5 **MCIG**[#] = 1.26 mM **Fingerprint endpoints** = eyes, heart, tail (78.4%, 75.7%, 78.4% of malformed embryos, respectively) **Hatching success at 76 hpf:** ↓ at 0.63 & 1.26 mM **Motility of unhatched embryos after dechorionation:** ↓ at 1.26 mM **Comet assay**: EC_{50} produced significant DNA damage compared to solvent control group

*TI = Teratogenic Index

[#] MCIG = Minimum Concentration Causing Growth Inhibition

Female Reproductive Toxicity of Chloroform in the Rat, Inhalation Route

Reference	Systemic Effects	Female Reproductive Effects
Schwetz et al., 1974	300 ppm: ↓ BW on GD-13 & 21 ↑ liver wt	300 ppm: ↓ pregnancy rate (3/20) ↓ litter size ↑ resorptions
Baeder & Hoffman, 1988	30 ppm: ↓ BW on GD-17	30 ppm: ↑ totally resorbed litters (early implantation)
Baeder & Hoffman, 1991	30 ppm: ↓ BW & wt gain	30 ppm: 1 lost litter No effect on litter size or resorption frequency

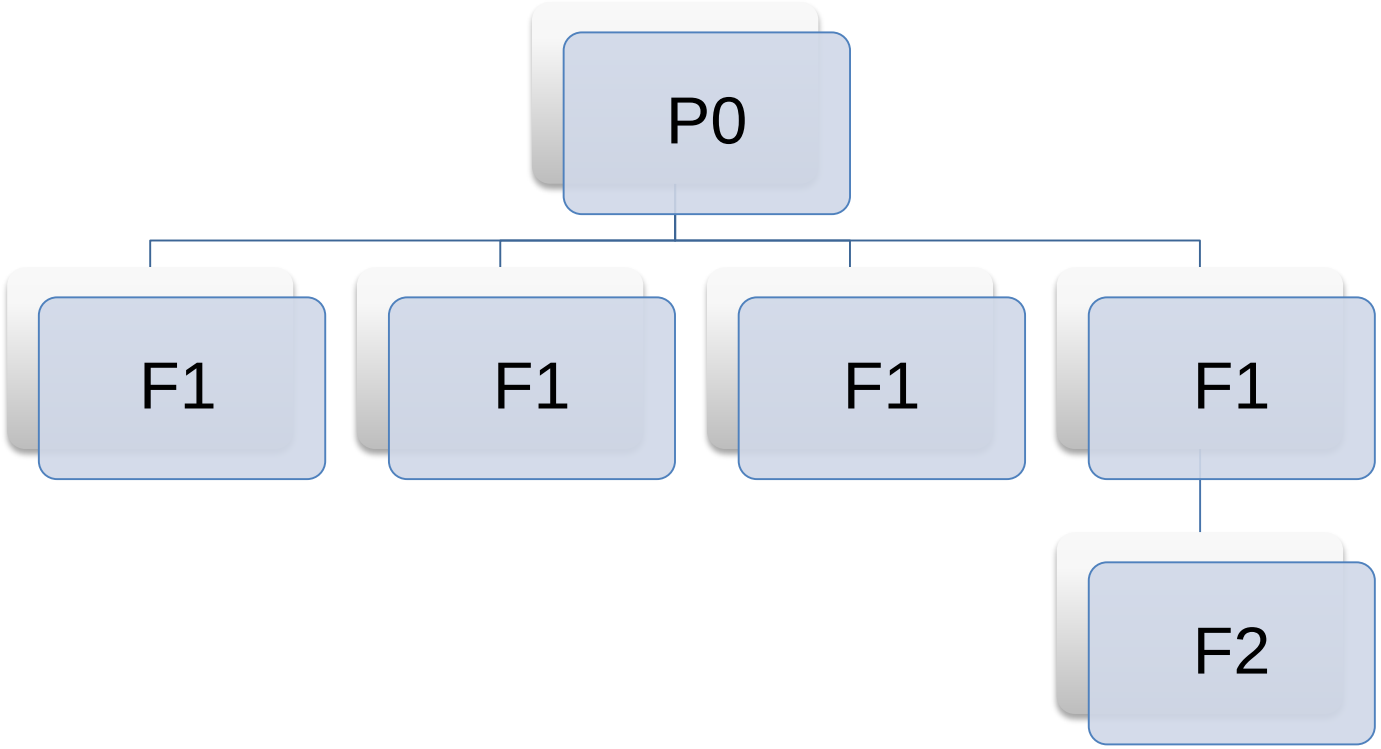
Female Reproductive Toxicity of Chloroform in the Mouse, Inhalation Route

Reference	Systemic Effects	Female Reproductive Effects
Murray et al., 1979	GD 6-15: 1/35 maternal death ↑ SGPT activity GD 1-7 or 8-15: ↓ wt gain, GD 6-15 or 8-15: ↑ absolute & relative liver wt	GD 1-7: ↑resorptions (2 litters completely resorbed) GD 1-7 or 6-15: ↓pregnancy rate

Female Reproductive Toxicity of Chloroform in the Rat, Oral Route

Reference	Systemic Effects	Female Reproductive Effects
Thompson et al, 1974 (range-finding study)	316 mg/kg-day: ↓ wt gain 1/6 Maternal deaths	316 mg/kg-day: ↑ resorptions ↓ litter size
Thompson et al, 1974 (full Teratology study)	126 mg/kg-day: Clinical symptoms ↓wt gain Fatty changes in livers	126 mg/kg-day: No clear evidence of female reproductive toxicity
Ruddick et al., 1983	400 mg/kg-day: ↓ wt gain ↑ liver wt ↑ kidney wt ↓red blood cell counts Altered blood chemistry	$\leq$ 400 mg/kg-day No effects on live litter size or resorption frequency

Continuous Breeding Protocol



Endpoints evaluated

- *Systemic*: clinical signs, BW, and water consumption.
- *Reproductive:* fertility, litters/pair, and sperm morphology or vaginal cytology at sacrifice

Female Reproductive Toxicity of Chloroform in the Mouse, Oral Route

Reference	Systemic Effects	Female Reproductive Effects
Chapin et al., 1977; NTP, 1988	41.2 mg/kg-day, P0: ↓ Maternal wt at delivery of 4th litter 41.2 mg/kg-day, F1 females: ↑ absolute & adjusted liver wt Minimal to moderate hepatocellular degeneration	41.2 mg/kg-day, F1: ↑ fertility index ↑ female pups/litter ↑ female + male pups/litter (no clear adverse effects)
US EPA, 1980	270 mg/kg-day ↑ fatty liver deaths	$\leq$ 270 mg/kg-day: No pathological changes noted for any group at any time in mammary, ovaries, or uterus

Female Reproductive Toxicity of Chloroform in the Rabbit, Oral Route

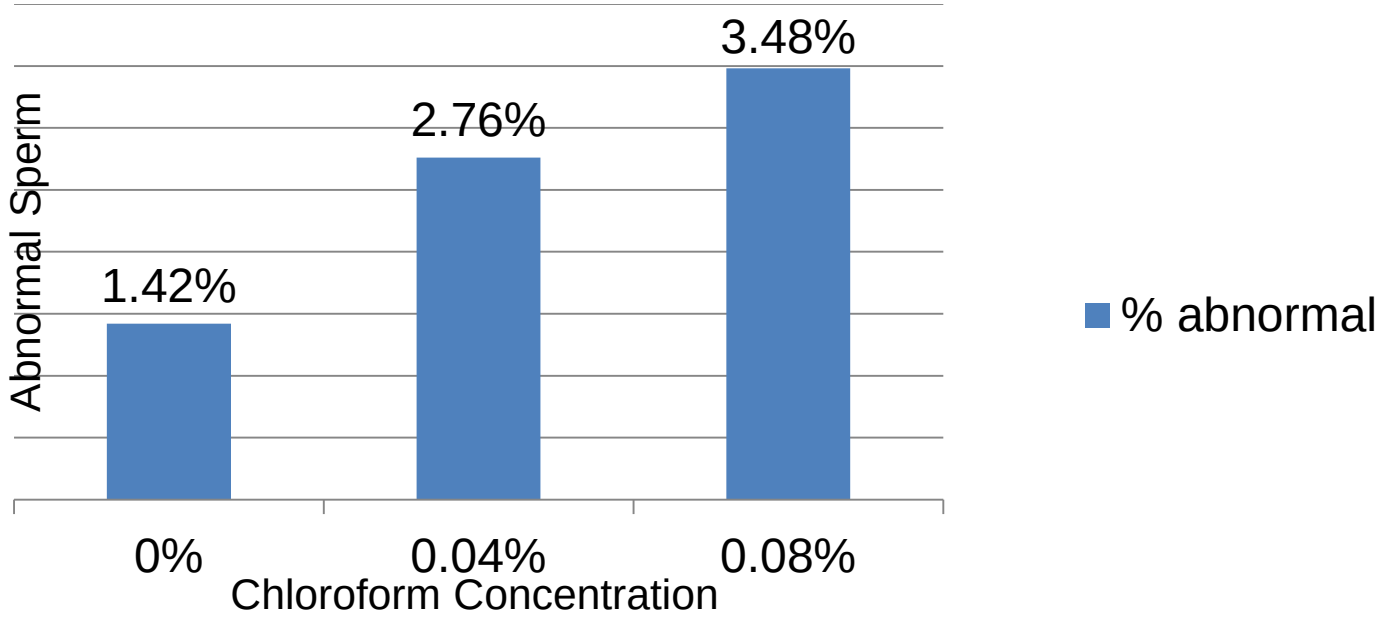
Reference	Systemic Effects	Female Reproductive Effects
Thompson et al., 1974 (range-finding study)	63 mg/kg-day: wt loss, 1/5 death	63 mg/kg-day: ↑ abortion of litters ↓ fetal viability in litters with live fetuses
Thompson et al., 1974 (full Teratology study)	All doses tested: Clinical symptoms during treatment	≤ 50 mg/kg-day: Aborted litters (no statistics or apparent dose response)

Female Reproductive Toxicity of Chloroform in Beagle Dogs, Oral Route

Reference	Systemic Effects	Female Reproductive Effects
Heywood et al., 1979	↑ biochemical indicators of liver damage	No treatment-related changes in ovaries or uteri

Male Reproductive Toxicity of Chloroform in the Mouse, Inhalation Route

Reference	Systemic Effects	Male Reproductive Effects
Land et al., 1981	Not discussed	Both treated groups: ↑ frequency of abnormal sperm morphology



Male Reproductive Toxicity of Chloroform in the Rat, Oral Route

Reference	Systemic Effects	Reproductive Effects
US EPA, 1980	160 mg/kg-day, all days: ↓ BW, also seen in water-matched controls	160 mg/kg-day, day 30 sacrifice: One case each of testicular hyperplasia and interstitial cell hyperplasia (not clear if single animal)

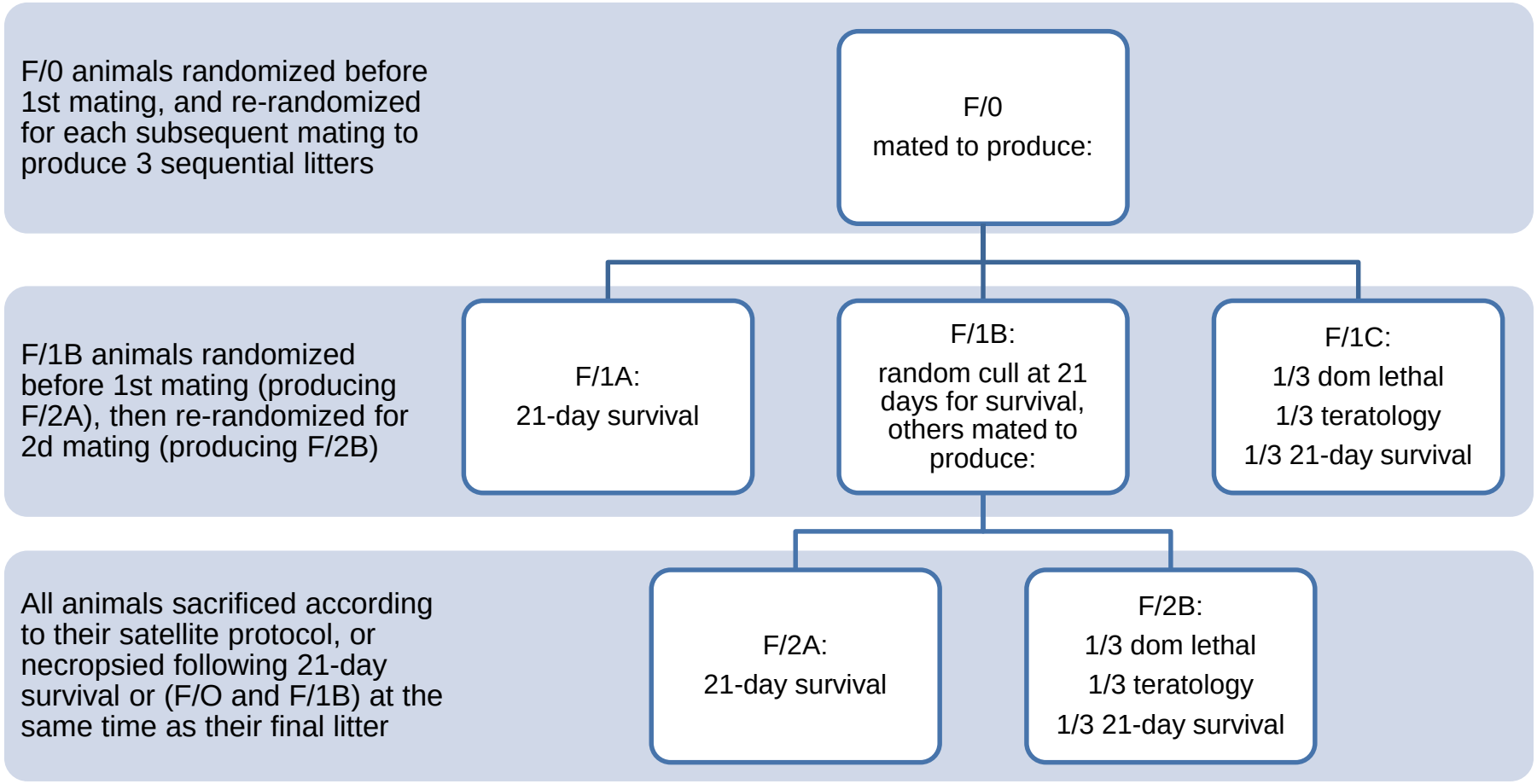
Male Reproductive Toxicity of Chloroform in the Mouse, Oral Route

Reference	Systemic Effects	Male Reproductive Effects
Chapin et al., 1977 & NTP, 1988	Not seen in males	41.2 mg/kg-day, P0: ↑ fertility index F1: ↑ fertility index ↑ Absolute right epididymal wt Minimal to mild degeneration of epididymal ductal epithelium (no clear adverse effects)

Male Reproductive Toxicity of Chloroform in Beagle Dogs, Oral Route

Reference	Systemic Effects	Reproductive Effects
Heywood et al., 1979	↑ biochemical indicators of liver damage	30 mg/kg-day: "Ectopic testes with inhibition of spermatogenesis" in 2 dogs (also 1 dog at 15 mg/kg-day, and 1 untreated control)

Protocol for Multigeneration Reproductive Toxicity Study With Satellite Components
Borzelleca and Carchman, 1982



Developmental and Reproductive Toxicity of Chloroform in a Multi-generation Mouse Study, Oral Route (Borzelleca & Carchman, 1982)

Maternal/Systemic Effects	Developmental/Reproductive Effects
↓ BW gain (♀ & ♂) in F/0 and F/1B at 5.0 mg/ml; F/1B ♀ at 1.0 mg/ml Enlarged livers, 5.0 mg/ml, F/0 and F/1B "almost all animals“ Final necropsies: liver pathology "characteristic of chlorinated hydrocarbon toxicity"	**Mating index**: ↓ at 0.1 mg/ml, F/1C; and at 5.0 mg/ml for F/1A, F/1B, and F/2A; but not F/1C or F/2B **Gestation index**: ↓ at 5.0 mg/ml: F/1A, F/1C, F/2A; but not for F/1B or F/2B **Viability Index**: ↓ (PND 4) at 1.0 mg/ml F/1B; and at 5.0 mg/ml in F/1A, F/1B, and F/2A litters **Lactation index**: ↓ at 1.0 mg/ml in F/1A litters; and at 5.0 mg/ml in F/1A and F/2A litters ↓ litter size at 5.0 mg/ml for F/1A, F/1B, F/1C, F/2A, and F/2B

Developmental and Reproductive Toxicity of Chloroform in a Multi-generation Mouse Study, Oral Route (Borzelleca & Carchman, 1982)

Part of Study	Developmental/Reproductive Effects
Dominant lethal satellites: F/1C & F/2B	No dominant lethal effect
Teratology satellites: F/1C & F/2B	No statistically significant effects of treatment on number of litters, implantations/dam, live fetuses/litter, % resorptions, or M/F ratio in F/1C or F/2B No effect of treatment on external or internal abnormalities from F/1C or F/2B No effect on skeletal abnormalities from F/1C (not assessed for F/2B)
21-day survival: all generations	Results used to calculate viability and lactation indices on previous slide; lowest effective concentration = 1.0 mg/ml

Summary of Developmental Toxicity Data in Animals

Species/Route	Inhalation	Oral	*In Vitro*
Rats	30 ppm	126 mg/kg-day	--
Mice	100 ppm	--	--
Rabbits	--	20 mg/kg-day	--
Zebrafish	--	--	0.63 mM

Summary of Female Reproductive Toxicity in Animals

Species/Route	Inhalation	Oral
Rats	30 ppm	NE* (400 mg/kg-day)
Mice	100 ppm	NE (270 mg/kg-day)
Rabbits	--	NE (63 mg/kg-day)
Dogs	--	NE (30 mg/kg-day)

*NE = No Observed Effect

Summary of Male Reproductive Toxicity in Animals

Species/Route	Inhalation	Oral
Rats	--	NE* (160 mg/kg-day)
Mice	0.04% in air	NE (41.2 mg/kg-day)
Dogs	--	NE (30 mg/kg-day)

Summary of DART Endpoints From Multi-generation Drinking Water Study in Rats

Endpoint	Concentration in Drinking Water
Developmental Toxicity	5.0 mg/ml
Female Reproductive Toxicity	0.1 mg/ml
Male Reproductive Toxicity	0.1 mg/ml

*NE = No Observed Effect