❖ T-modality

1) Completeness of the database: is the dataset complete for the ED assessment in line with the ED GD?

T-mediated adversity has been investigated in OECD TG 408 (ID: 1, 2, 4), 409 (ID: 6), 451 (ID: 5a, 5b), 452 (ID: 7), 453 (ID: 3a, 3b) studies.

More specifically:

- **Thyroid weight** has been investigated in 90-day (ID: 6) and 1-year (ID: 7) dog studies. Thyroid weight is not measured in rats and mice.
- **Thyroid histopathology** has been investigated in short-term and long-term studies in rats (ID: 1, 2, 3a, 3b), dogs (ID: 6, 7) and mice (4, 5a).

There is no OECD TG 407 study included in the dossier. Thyroid adversity is not measured in the available OECD TG 416 (ID: 8) study.

Thyroid hormone measurements are not included in any of the available studies.

An outline on the available data is presented in Table 1, below:

Table 1. Sufficient data set for T-mediated adversity

Species	Duration	Guideline	Study ID	Thyroid weight	Thyroid histopathology	Hormone measurements
rat	90-day	OECD TG 408	1	No data	✓	No data
			2	No data	✓	No data
	12-months	OECD TG 453	3a	No data	✓	No data
	22-months	OECD TG 453	3b	No data	✓	No data
	2-generation	OECD TG 416	8	No data	No data	No data
dog	3-months	OECD TG 409	6	✓	✓	No data
	1-year	OECD TG 452	7	✓	✓	No data
mouse	90-day	OECD TG 408	4	No data	✓	No data
	24-months	OECD TG 451	5a	No data	✓	No data
	12-months	OECD TG 451	5b	No data	No data	No data

With regard to sufficiency of T (thyroid) dataset reference is made to the EFSA Technical report on the outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology (EFSA supporting publication 2020:EN-1837):

- Regarding the availability of THs (thyroid hormones) measurements to evaluate the sufficiency of the adversity related to the T-modality:
  "*EFSA clarified that in the old versions of the OECD TGs the measurement of thyroid hormones was optional. Therefore, in these cases, the lack of THs measurement cannot be used to conclude that the dataset for adversity is not complete. However, it should be noted that in the new versions of OECD TGs, THs measurement is mandatory.*"
- *Moreover, "the dataset for thyroid can be considered complete on a case-by-case basis, pending whether the duration and doses selection allow a proper assessment of*

the thyroid histology (thyroid histopathology is generally considered more sensitive and informative than thyroid weight).

2) Have T-mediated patterns of adversity been observed?

3) Where adverse effects observed at doses considered to overcome the MTD or be considered too high (exaggerated) based on the overall toxicity profile?

4) If a convincing *pattern of adverse effect/s* for T mediated parameters can be drawn, the MoA analysis should be performed. However, following a coherence analysis, in most of the cases, the work can be finalized, and ED criteria are met.

5) Are there in-vitro and/or in-vivo mechanistic data? If yes, do they provide positive or negative findings?

Overall conclusion for T-modality:

❖ EAS-modalities

1) Completeness of the database: is the dataset complete for the ED assessment in line with the ED GD?

Overall, the following parameters were not investigated:

- Estrus cycle
- Sperm parameters (sperm count, sperm motility, sperm morphology)
- Age at vaginal opening
- Age at preputial separation
- Quantitative evaluation of primordial follicles of the ovaries
- Epididymis weight
- Ovary weight
- Uterus weight
- Prostate weight
- Seminal vesicles weight
- Coagulating gland weight

2) **Have EATS mediated patterns of adversity been observed?**

3) **Where adverse effects observed at doses considered to overcome the MTD or be considered too high (exaggerated) based on the overall toxicity profile?**

4) **If a convincing *pattern of adverse effect/s* for EATS mediated parameters can be drawn, the MoA analysis should be performed. However, following a coherence analysis, in most of the cases, the work can be finalized, and ED criteria are met.**

5) **Are there in-vitro and/or in-vivo mechanistic data? If yes, do they provide positive or negative findings?**

EAS effects & systemic toxicity

Rat studies:

- **90-day (feeding) – ID: 1**

(Doses: 6.56/7.71, 133/153, 658/783, 1036/1124 mkd in M/F)

Study NOAEL = 6.6/7.7 mkd

At 1036/1124 mkd (15000 ppm):

EAS parameters:

- **↑ Mean relative testes weight (↑ 29% stat signif). ↑ Absolute testis weight (5% not stat signif)**
- **No effect on:**
  - Vagina histopathology
  - Uterus histopathology (with cervix)
  - Thyroid histopathology
  - Testis histopathology
  - Seminal vesicles histopathology
  - Prostate histopathology (with seminal vesicles and coagulating glands)
  - Ovary histopathology
  - Mammary gland histopathology (female)
  - Epididymis histopathology

Systemic toxicity:

- **Body weight:** ↓ body weight in M **(↓ 19% stat signif)** and F (17% stat signif)
- **Food consumption:** ↓ food consumption in M **(↓ 10% stat signif)** and F (13% stat signif)
- **Food efficiency (0-91 days):** ↓ food efficiency in M (↓ 19% stat signif) and F (20% stat signif)
- **Spleen weight:** stat signif. ↑ in rel-to-body spleen weight in M (↑31%) and F (↑30%)
- **Spleen histopathology:** Extramedullary hematopoiesis in M (9/10 vs 3/10 in controls) and F (7/10 vs 0/10 in controls)
- **Liver weight:** stat signif. ↑ in rel-to-body liver weight in M (↑20%) and F (↑28%)
- **Kidney weight:** stat signif. ↑ in rel-to-body kidney weight in M (↑12%) → in females there is ↑ 8.8% not stat signif
- **Kidney histopathology:** hemosiderosis (pigment in proximal tubules) in M (4/10 vs 0/10) and F (6/10 vs 0/10)
- **Heart weight:** stat signif. ↑ in rel-to-body heart weight in M (↑24%) and F (↑24%)
- **Clinical chemistry and haematology:**
  - ↑ in MCV in M **(↑ 9%, stat signif)** and F (↑ 5% stat signif)
  - ↑ reticulocyte count in M **(↑53% stat signif) and F (↑ 230% stat signif)**
  - Lymphocytosis: ↑ lymphocytes in M (↑ 39%). No effect in F.

Brain weight: stat signif. ↑ in rel-to-body brain weight in M (↑20%) and F (↑24%).

At 658/783 mkd (10000 ppm):

EAS parameters:

- **↑ Mean relative testes weight (↑ 27% stat signif). ↑ absolute testes weight (↑ 12% stat signif)**
- **No measurements on:**
  - Vagina histopathology
  - Uterus histopathology (with cervix)
  - Thyroid histopathology
  - Testis histopathology
  - Seminal vesicles histopathology

- Prostate histopathology (with seminal vesicles and coagulating glands)
- Ovary histopathology
- Mammary gland histopathology (female)
- Epididymis histopathology

Systemic toxicity:

- **Body weight:** ↓ body weight in M **(↓ 12% stat signif)** and F (14% stat signif)
- **Food consumption (0-91 days):** ↓ food consumption in F **(13% stat signif)**; no effect in males
- **Food efficiency (0-91 days):** ↓ food efficiency in M (↓ 19% stat signif) and F (20% stat signif)
- **Spleen weight:** stat signif. ↑ in rel-to-body spleen weight in M (↑22%) and F (↑24%)
- **Spleen histopathology:** Extramedullary hematopoiesis in M (9/10 vs 3/10 in controls) and F (4/10 vs 0/10 in controls)
- **Liver weight:** stat signif. ↑ in rel-to-body liver weight in F (↑33%). No effect in males.
- **Kidney weight:** No stat signif changes.
  ↑ in rel-to-body kidney weight in F (↑9.3%). No effect in males.
- **Kidney histopathology:** hemosiderosis (pigment in proximal tubules) in M (3/10 vs 0/10) and F (2/10 vs 0/10)
- **Heart weight:** stat signif. ↑ in rel-to-body heart weight in M (↑14%) and F (↑15%)
- **Clinical chemistry and haematology:**
  - ↑ in MCV in M **(↑ 5%, stat signif)**; no stat signif ↑ in females
  - ↑ reticulocyte count in M **(↑72% stat signif)** and F (↑ 182% stat signif)
  - Lymphocytosis: ↑ lymphocytes in M (↑ 37%). No effect in F.

Brain weight: stat signif. ↑ in rel-to-body brain weight in M (↑13%) and F (↑23%).

<u>At 133/153 mkd (2000 ppm):</u>

EAS parameters:

↑ Mean relative testes weight (↑ 10% non stat signif)

- **No measurements on:**
  - Vagina histopathology
  - Uterus histopathology (with cervix)
  - Thyroid histopathology
  - Testis histopathology
  - Seminal vesicles histopathology
  - Prostate histopathology (with seminal vesicles and coagulating glands)
  - Ovary histopathology
  - Mammary gland histopathology (female)
  - Epididymis histopathology

Systemic toxicity:

- **Body weight:** ↓ body weight in M **(↓ 7%)** and F (3%)
- **Food consumption (0-91 days):** no effect
- **Food efficiency (0-91 days):** ↓ food efficiency in M (↓ 10% stat signif); no effect in females
- **Spleen histopathology:** Extramedullary hematopoiesis in F (2/10 vs 0/10 in controls). No effect in males.
- **Liver weight:** stat signif. ↑ in rel-to-body liver weight in F (↑11%). No effect in males.

- **90-day (feeding) – ID: 2**

(Doses: 6.20/7.54, 127/150, 646/774, 965/1070 mkd in M/F**)**

At 965/1070 mkd:

EAS parameters:

- **Testis weight:** ↓ absolute testes weight **(↓ 31% stat signif).**
- **Testis histopathology: Atrophy, degeneration, bilateral Leydig cell hyperplasia**
  In RAR also gross observations: small testes (7/10 vs 0/10 in controls)
- **Epididymis histopathology: Oligospermia, atrophy**

(ppm):	**0**	**100**	**2000**	**10000**	**15000**
(mkd):	**0**	**6.2**	**127**	**646**	**965**
Number of rats/group:	**10**	**10**	**10**	**10**	**10**
Testes:					
Gross observation: small testes	0	0	0	1	7
Atrophy/degeneration, seminiferous tubules, bilateral	**1**	0	0	**2**	**8**
Atrophy/degeneration, seminiferous tubules, unilateral	0	0	**2**	**1**	**1**
Dilatation, lumen, seminiferous tubules	0	0	0	0	**1**
Hyperplasia, Leydig cell, bilateral	0	0	0	0	**7**
Hyperplasia, Leydig cell, unilateral	0	0	0	**1**	0
Epididymides:					
Oligospermia, bilateral	**1**	0	0	**2**	**8**
Oligospermia, unilateral	0	0	**1**	**1**	**1**
Sperm granuloma	0	0	0	**1**	**1**

- **No effect on:**
  - Vagina histopathology
  - Uterus histopathology (with cervix)
  - Thyroid histopathology
  - Seminal vesicles histopathology
  - Prostate histopathology (with seminal vesicles and coagulating glands)
  - Ovary histopathology
  - Mammary gland histopathology (female)

Systemic toxicity:

- **Body weight:** ↓ body weight in M **(↓ 40% stat signif**) and F (↓35% stat signif)
- **Body weight gain (0-91 days):** ↓ body weight gain in M **(↓ 72% stat signif**) and F (↓82% stat signif)
- **Food consumption (0-91 days):** ↓ food consumption in M (34% stat signif) and F (↓19% stat signif)
- **Food efficiency (0-91 days):** ↓ food efficiency in M (↓ 56.8% stat signif) and F (↓73% stat signif)
- **Spleen weight:** ↑ in rel-to-body spleen weight in M (↑42% stat signif.) and F (↑36% stat signif.)
- **Liver weight:** ↑ in rel-to-body liver weight in M (↑13% stat signif.) and F (↑34% stat signif.)
- **Kidney weight:** ↓ in absolute kidney weight in M (↓34% stat signif.) and F (↓ 22% stat signif.) Relative-to-body kidney weight was increased in F (↑ 20% stat signif). No effect in males.
- **Kidney histopathology:** hemosiderosis (pigment in proximal tubules) in M (2/10 vs 0/10) and F (6/10 vs 0/10); tubular epithelial cell atrophy in M (1/10 vs 0/10) and F (9/10 vs 0/10).
- **Clinical chemistry and haematology:**
  - ↓ in RBC count in M **(↓ 18%, stat signif**) and F (↓ 16%, stat signif)
  - ↓ in Hb in M **(↓ 10%, stat signif**) and F (↓ 11%, stat signif)
  - ↓ in haematocrit in M **(↓ 7.5%, stat signif**) and F (↓ 11%, stat signif)
  - ↑ MCV in M **(↑13% stat signif**) and F (↑ 6 % stat signif)
  - ↑ reticulocyte count in M **(↑342% stat signif**) and F (↑ 91% stat signif)

Brain weight: ↓ absolute brain weight in M (↓ 9% stat signif). ↑ in rel-to-body brain weight in M (↑51%) and F (↑48%).

At 646/774 mkd:

EAS parameters:

(ppm):	**0**	**100**	**2000**	**10000**	**15000**
(mkd):	**0**	**6.2**	**127**	**646**	**965**
Number of rats/group:	**10**	**10**	**10**	**10**	**10**
Testes:					
Gross observation: small testes	0	0	0	1	7
Atrophy/degeneration, seminiferous tubules, bilateral	1	0	0	2	8
Atrophy/degeneration, seminiferous tubules, unilateral	0	0	2	1	1
Atrophy/degeneration, seminiferous tubules, bilateral or unilateral	1	0	2	**3**	9
Dilatation, lumen, seminiferous tubules	0	0	0	0	1
Hyperplasia, Leydig cell, bilateral	0	0	0	0	7
Hyperplasia, Leydig cell, unilateral	0	0	0	1	0
Epididymides:					
Oligospermia, bilateral	1	0	0	2	8
Oligospermia, unilateral	0	0	1	1	1
Oligospermia, bilateral or unilateral	1	0	1	**3**	9
Sperm granuloma	0	0	0	**1**	1

- **No measurement on:**
  - Vagina histopathology
  - Uterus histopathology (with cervix)
  - Thyroid histopathology
  - Seminal vesicles histopathology
  - Prostate histopathology (with seminal vesicles and coagulating glands)
  - Ovary histopathology
  - Mammary gland histopathology (female)

Systemic toxicity:

- **Body weight:** ↓ body weight in M **(↓ 30% stat signif**) and F (29% stat signif)
- **Body weight gain (0-91 days):** ↓ body weight gain in M **(↓ 52% stat signif**) and F (↓67% stat signif)
- **Food consumption (0-91 days):** ↓ food consumption in M (24% stat signif) and F (↓19% stat signif)
- **Food efficiency (0-91 days):** ↓ food efficiency in M (↓ 38% stat signif) and F (↓59% stat signif)
- **Spleen weight:** ↑ in rel-to-body spleen weight in M (↑38% stat signif.) and F (↑29% stat signif.)
- **Liver weight:** ↑ in rel-to-body liver weight in M (↑5% stat signif.) and F (↑32% stat signif.)
- **Kidney weight:** ↓ in absolute kidney weight in M (↓21% stat signif.) and F (↓ 16% stat signif.) Relative-to-body kidney weight was increased in M (↑ 11% stat signif) and F (↑ 17% stat signif)
- **Kidney histopathology:** hemosiderosis (pigment in proximal tubules) in M (6/10 vs 0/10) and F (6/10 vs 0/10).
- **Clinical chemistry and haematology:**
  - ↓ in RBC count in M **(↓ 16%, stat signif**) and F (↓ 16%, stat signif)
  - ↓ in Hb in M **(↓ 10%, stat signif**) and F (↓ 11%, stat signif)
  - ↓ in haematocrit in M (↓ **7.4%, stat signif**) and F (↓ 11%, stat signif)
  - ↑ MCV in M (↑8% stat signif) and F (↑ 6 % stat signif)
  - ↑ reticulocyte count in M **(↑288% stat signif**) and F (↑ 91% stat signif)

Brain weight: ↓ absolute brain weight in M (↓ 7% stat signif). ↑ in rel-to-body brain weight in M (↑31%) and F (↑37%).

At 127/150 mkd: no EAS effects (LOAEL)

- **2-generation (feeding) – ID: 8; study conducted in 1990-1991 prior to the OECD TG 416 (2001).**

(Doses: 0.588 mkd, 5.81 mkd, 44.0 mkd, 89.5 mkd

At the highest dose 89.5 mkd:

EAS effects:

- **Testis weight**
  ↑ relative testis weight in F0 (10%, stat. sign) and F1 (16%, stat. sign.). No effect in abs. testes weight.

	0 ppm	10 ppm	100 ppm	750 ppm	1500 ppm
F_0 Males	**0 mkd**	**0.588 mkd**	**5.81 mkd**	**44.0 mkd**	**89.5 mkd**
Mean final body weight (g)	654.0	636.7	638.2	612.7*	591.3*
Absolute testes weight (g)	3.729	3.591	3.505*(↓6 %)	3.719	3.670
Relative[a] testes weight	0.5718	0.5686	0.5524	0.6079* ↑6%	0.6267* ↑10%
F_1 Males	**0 mkd**	**0.785 mkd**	**7.84 mkd**	**59.6 mkd**	**123.0 mkd**
Mean final body weight (g)	691.7	681.3	738.4	647.6	619.8*
Absolute testes weight (g)	3.974	4.040	4.016	3.856	4.127
Relative[a] testes weight	0.5798	0.6006	0.5477	0.6016	0.6748* ↑16%

[a] Relative weight is defined as the organ to body weight ratio.

* Significantly different from control by the Dunnett's criteria, p <0.05.

- **Testis histopathology**
  No effect in F0 and F1 (control and high dose only)
- **Prostate histopathology**
  No effect in F0 and F1 (control and high dose only)
- **Epididymis histopathology**
  No effect in F0 and F1 (control and high dose only)
- **Seminal vesicles histopathology**
  No effect in F0 and F1 (control and high dose only) (RAR, not in excel)
- **Coagulating gland histopathology**
  No effect in F0 and F1 (control and high dose only) (RAR, not in excel)
- **Vagina histopathology**
  No effect in F0 and F1 (control and high dose only)
- **Uterus histopathology**
  No effect in F0 and F1 (control and high dose only)
- **Ovary histopathology**
  No effect in F0 and F1 (control and high dose only)
- **Pituitary histopathology**
  No effect in F0 and F1 (control and high dose only)

Systemic toxicity:

- ↓ food consumption (6.2%, stat. sign) and food efficiency (17%, stat. sign) in M (F0)
- ↓ food efficiency (14.5%, stat. sign) in F (F0) during premating

- ↓food efficiency (6%, stat. sign) in M (F1) during premating
- ↓ food consumption (6.7%), stat. sign) during premating in F (F1)
- F0: **↓ BW (10%, stat. sign in M) & BW gain (22%, stat. sign. in M)**
- F0: ↓BW during lactation (6%, stat. sign.) and gestation (6%, stat. sign) & ↓BW gain during premating (18%, stat. sign)
- F1: **↓BW & BW gain during premating (10%, stat. sign)**
- F1: ↓BW during premating, lact., gest. (10%, 9%, 8%, respectively, all stat. sign.) and ↓BW gain (11%, stat. sign) during premating.
- No effect in reproductive parameters. There were no biologically or statistically significant differences in mating indices, fertility indices, or gestation length in any of the F0 or F1 treatment groups.
- ↓ F1 offspring: Pup weight (7%, stat. sign)

Body weight/nutritional parameters in F_0 parental rats

	0 ppm	**10 ppm**	**100 ppm**	**750 ppm**	**1500 ppm**
Males (premating; Day 0-70):	**0 mkd**	**0.588 mkd**	**5.81 mkd**	**44.0 mkd**	**89.5 mkd**
Body weight (g)	590.4	572.0	573.1	558.3* **↓5%**	532.4* **↓10%**
Body weight gain (g)	238.4	222.8	221.6	207.5* **↓13%**	185.5* **↓22%**
Food consumption (g)	29.1	28.4	28.1	27.9	27.3* **(↓6.2%)**
Food efficiency[a]	0.117	0.111	0.112	0.106* **(↓1.1%)**	0.097* **(↓2%)**
Females (premating/gestation):	**0 mkd**	**0.764/0.741 mkd**	**7.75/7.80 mkd**	**58.0/55.6 mkd**	**115.0/114.0 mkd**
Body weight (g)					
Premating Day 70	308.1	307.7	312.5	293.6	293.4
Gestation Day 21	470.9	458.1	471.7	444.9	441.8* ↓6%
Lactation Day 21	353.4	351.5	353.0	327.9* ↓7%	333.5* ↓6%
Body weight gain (g)					
Premating Day 0-70	102.0	99.7	104.6	87.8* ↓14%	83.3* ↓18%
Gestation Day 0-21	153.3	147.5	159.3	146.5	148.5
Lactation Day 0-21	-1.1	-1.0	-1.2	4.0	6.0
Food consumption (g)					
Premating Day 0-70	21.1	20.7	21.3	20.1	20.2
Gestation Day 0-14	27.8	26.5	28.4	25.2*	25.3
Food efficiency[a]					
Premating Day 0-70	0.069	0.069	0.070	0.062* (↓10.1%)	0.059* (↓14.5%)
Gestation Day 0-14	0.168	0.167	0.175	0.164	0.159

[a] Calculated as g weight gain/g food consumed.

* Significantly different from control by Dunnett's criteria, $p < 0.05$.

Two-generation reproduction study: Body weight/nutritional parameters in F_1 parental rats

	0 mkd	0.588 mkd	5.81 mkd	44.0 mkd	89.5 mkd
Males (premating; Day 0–105):					
Body weight (g)	606.5	600.9	644.2	572.5	546.9* **↓10%**
Body weight gain (g)	552.3	546.6	587.7	521.2	495.7* ↓10%
Food consumption (g)	28.6	28.4	30.1	27.7	27.3
Food efficiency[a]	0.184	0.182	0.187	0.180	0.173* **(↓6%)**
Females:					
Body weight (g)					
Premating Day 105	329.6	334.7	337.9	307.8* ↓7%	295.3* ↓10%
Gestation Day 21	498.0	484.1	486.7	464.3* ↓7%	452.1* ↓9%
Lactation Day 21	367.6	364.2	367.4	343.4* ↓7%	336.5* ↓8%
Body weight gain (g)					
Premating Day 0-105	276.3	282.1	284.4	258.2 ↓7%	246.0* ↓11%
Gestation Day 0-21	165.1	150.5	143.1	155.5	149.7
Lactation Day 0-21	-16.1	-13.8	-13.5	-6.0	-2.8
Food consumption, (g)					
Premating Day 0-105	20.9	21.0	21.4	19.8* (↓5.3%)	19.5* (↓6.7%)
Gestation Day 0-14	26.9	26.1	27.2	25.6	24.7
Food efficiency[a]					
Premating Day 0-105	0.126	0.128	0.126	0.125	0.121
Gestation Day 0-14	0.201	0.183	0.163	0.177	0.176

[a] Calculated as g weight gain/g food consumed.

* Significantly different from control by the Dunnett's criteria, p <0.05.

At 44 mkd:

- **↑ (6%, stat. sign.) in relative testis weight in F0. No effect in abs. testis weight. No effect in F1.**

Systemic toxicity:

- ↓ food consumption (4%) and food efficiency (9%, stat. sign) in M (F0)
- F0: **↓ BW (5%, stat. sign in M) & BW gain (13%, stat. sign. in M)**
- F0: ↓BW in F during lactation (7%, stat. sign.) & ↓BW gain during premating (14%, stat. sign)
- No effect in reproductive parameters. There were no biologically or statistically significant differences in mating indices, fertility indices, or gestation length in any of the F0 or F1 treatment groups.

Combined chronic toxicity/oncogenicity study, 2-year feeding study in rats with interim sacrifice (ID:3a & 3b)

The study was terminated after 22 months due to poor survival.

Deviations from OECD 453 (2018):

- Weights of epididymis, thyroids, uterus, ovaries were not measured.
- The following tissues were not collected: cervix, coagulation glands.

Doses: 0.406/0.546 mkd, 4.06/5.47 mkd, 30.6/41.5 mkd, and 64.5/87.7 mkd (M/F)

At 64.5/87.7 mkd:

EAS effects

- **Epididymis histopathology:**
  No effect
- **Mammary gland histopathology:**
  ↓ incidence of mammary masses (fibroadenomas) in F at terminal sacrifice (30/50 vs 44/48 in control)
- **Ovary histopathology:**
  No effect. Control and high dose only
- **Prostate histopathology:**
  No effect. Control and high dose only
- **Seminal vesicles histopathology:**
  No effect. Control and high dose only
- **Testis weight**
  ↑ rel. testis weight (28%, stat. sign) and ↑ abs. testis weight (6%, not stat. sign) at interim sacrifice
  ↑ rel. testis weight (23%, stat. sign) and ↑ abs. testis weight (5%, not stat. sign) at terminal sacrifice

Parameter	0 mkd	0.406 mkd	4.06 mkd	30.6 mkd	64.5 mkd
Males:					
12-Month					
Absolute testes weight (g)	3.803	3.710	3.776	3.784	4.047
Relative testes weight	0.4571	0.4493	0.4764	0.5221 **↑14%**	0.5856[b] **↑28%**
24-Month					
Absolute testes weight (g)	3.708	3.668	3.706	3.768	3.906
Relative testes weight	0.4730	0.5172	0.5203	0.4943	0.5822[b] **↑23%**

[a] Relative weight is defined as the organ to body weight ratio.

[b] Significantly different from control by the Dunnett's test criteria, p <0.05.

- **Testis histopathology**

	0 mkd	0.406 mkd	4.06 mkd	30.6 mkd	64.5 mkd

Males:					
Testes					
Number examined	51	46	47	50	51
Adenoma, interstitial cell	0 (0%)	2 (4.3%)	1 (2.1%)	**7* (14.0%)**	**7* (13.7%)**
Hyperplasia, interstitial cell	10 (19.6%)	7 (15.2%)	11 (23.4%)	**18* (36.0%)**	**27* (52.9%)**
Combined adenoma and hyperplasia	10	9	12	**25**	**34**

* Statistically significant ($p \leq 0.05$) by the Cochran-Armitage test

- **Uterus histopathology:**
  No effect. Control and high dose only
- **Vagina histopathology:**
  No effect. Control and high dose only

Systemic toxicity:

- ↓ RBC count (**17%, stat. sign**) , ↓ Hb (14%, stat. sign), ↓ Ht (14%, stat. sign) at interim sacrifice in M
- ↓ (stat. sign.) **BW (14% in M**, 16% in F) & BW gain (20% in M, 28% in F) at interim sacrifice
- ↓ (stat. sign.) in **BW (14% in M,** 15% in F) & BW gain (20% in M, 23% in F) at terminal sacrifice
- ↓ in triglyceride conc (46%, stat. sign) at interim sacrifice.
- 54% survival for M and 46% for F. According to the study author, the poor survival rate is not a treatment related effect but typical for this strain of rat (Crl:CD® BR)
- ↑ in rel. liver weight (13%, stat. sign) in F at interim sacrifice
- ↑ rel. brain weight (19%, stat. sign.) in M and 15% (stat. sign) in F at interim sacrifice and ↑ 16% stat. sign) in F at terminal sacrifice
- ↓abs. kidney weight (14%, stat. sign), abs. spleen weight (19%, stat. sign) in F at terminal sacrifice

Concentration	0 ppm	10 ppm	100 ppm	750 ppm	1500 ppm
12 months					
Males:	**0 mkd**	**0.406 mkd**	**4.06 mkd**	**30.6 mkd**	**64.5 mkd**
Mean body weight	819.4	784.8	822.1	759.1* **↓7%**	706.8* **↓14%**
Body weight gain	568.7	535.3	570.7	506.6* **↓11%**	456.4* **↓20%**
Females	**0 mkd**	**0.546 mkd**	**5.47 mkd**	**41.5 mkd**	**87.7 mkd**
Mean body weight	418.4	412.1	417.8	387.6* ↓7%	350.5* ↓16%
Body weight gain	241.7	235.7	239.7	210.4* ↓13%	174.0* ↓28%
22 Months					
Males:	**0 mkd**	**0.406 mkd**	**4.06 mkd**	**30.6 mkd**	**64.5 mkd**
Mean body weight	827.2	758.5	768.7	782.8	712.8* **↓14%**
Body weight gain	578.1	512.2	519.1	533.1	463.3* **↓20%**
Females	**0 mkd**	**0.546 mkd**	**5.47 mkd**	**41.5 mkd**	**87.7 mkd**
Mean body weight	463.9	495.6	485.3	454.5	396.3* ↓15%
Body weight gain	287.2	318.8	313.1	279.6	220.0*

					↓23%

* Significantly different from control by the Dunnett's criteria, p <0.05.

- No effect in pituitary-, brain-, adrenal- histopathology,

At 30.6/41.5 mkd:

EAS effects

- **Epididymis histopathology:**
  No effect
- **Mammary gland histopathology:**
  ↓ incidence of mammary masses (fibroadenomas) in F at terminal sacrifice (37/48 vs 44/48 in control)
- **Testis weight**
  ↑ rel. testis weight (14%, not stat. sign) at interim sacrifice
  No effect at terminal sacrifice
- **Testis histopathology**
  No effect at interim sacrifice
  ↑ Leydig cell hyperplasia (36.0%, stat. sign) and ↑ Leydig cell adenoma (14%, stat. sign) at terminal sacrifice. Outside HC data.

Systemic toxicity:

- ↓ RBC count (**10%, stat. sign**), ↓ Hb (8%, stat. sign), ↓ Ht (7%, stat. sign) at interim sacrifice in M
- ↓ (stat. sign.) BW (7% in M, 7% in F) & BW gain (11% in M, 13% in F) at interim sacrifice
- No effect in BW and BW gain in both M&F (<10%) at terminal sacrifice
- ↓ in triglyceride conc (30%, not stat. sign) at interim sacrifice. Not biologically adverse effect
- 41% survival for M and 35% for F. According to the study author, the poor survival rate is not a treatment related effect but typical for this strain of rat (Crl:CD® BR)

Mouse studies:

- **18-month (feeding) – ID: 5**

(Doses: 1.37/1.86 mkd, 20.9/27.7 mkd, 349/488 mld, and 1024/1360 mkd (M/F))

Deviations from OECD 451 (2018) : cervix and coagulating glands were not collected

At 1024/1360 mkd:

EAS effects

No effect in:

Vagina histopathology

Uterus histopathology

Ovary histopathology

Mammary gland histopathology

Testis weight and histopathology

Seminal vesicles histopathology

Prostate histopathology

Epididymis histopathology

Systemic toxicity:

- ↓BW gain (11% in M, 16%, stat. sign in F)

Dog studies:

- **3-month (feeding) – ID: 6**

(Doses: 3.90/3.70, 146/160, and 268/251 mkd in M/F)

At 268/251 mkd in M/F:

EAS parameters:

- **Testis weight: ↓ absolute testes weight**
- **Testis histopathology: Bilateral tubular atrophy, decrease thickness of the seminiferous tubules, cytoplasmic vacuolation of germinal epithelium**
- **Epididymis histopathology: Aspermatogenesis, oligospermia**

(ppm):	**0**	**100**	**4000**	**8000**
(mkd):	**0**	**3.9**	**146**	**268**
Number of dogs/group:	**4**	**4**	**4**	**4**
Organ weights **[Note: Confirm that there are no separate measurements on testes and epididymides weight in original study report (page 240)]**				
Absolute testes/epididymides weight	16.3	15.8	12.3 ↓25%	7.5** ↓64%
Relative[a] (to body weight) testes/epididymides weight	1.46	1.60	1.12 ↓23%	0.81* ↓45%
Relative[b] (to brain weight) testes/epididymides weight	2.04	2.06	1.56 ↓24%	0.97* ↓52%
Incidences of microscopic effects				
Testis				
Small (gross lesion)	0	0	2	4
Tubular atrophy, bilateral	0	0	1	3
Tubular atrophy, unilateral	0	0	1	0
Germinal epithelium: cytoplasmic vacuolation	0	0	0	2
overall			**2**	**3**
Epididymides				
Small (gross lesion)	0	0	0	2
Aspermatogenesis	0	0	1	3
Oligospermia	0	0	**1**	**1**
Cell debris	0	1	4	3

- **No effect on:**
  - Uterus histopathology (with cervix)
  - Thyroid weight
  - Thyroid histopathology
  - Ovary histopathology

Systemic toxicity:

- **Mortality:** 2/4 females became emaciated and during the study and were sacrificed *in extremis* on days 76 & 78.
- **Body weight:** **↓ body weight in M (↓ 25% stat signif**) and F (↓ 15% stat signif)
- **Body weight gain (0-13 weeks):** **↓ body weight gain in M (↓ 83% stat signif**) and F (57% stat signif)
- **Food consumption (0-13 weeks):** **overall no significant effect**
  (Food consumption slightly decreased during the 1st month of the study due to poor palatability corrected for the remaining of the study & food consumption was not affected.)
- **Liver weight:** **↑ in rel-to-body liver weight in M (↑60% stat signif.)** and F (↑55% stat signif.)
- **Liver histopathology:** Bile stasis (f); Pigmented sinusoidal macrophages (m&f)
- **Bone marrow:** hypercellularity (RMS: this is indicative of regenerative anaemia, see also haematology changes).

(ppm):	**0**	**100**	**4000**	**8000**
(mkd):	**0**	**3.9**	**146**	**268**
Number of dogs/group:	**4**	**4**	**4**	**4**
Males				
Liver	0	0	4	3
Enlarged (gross lesion)				
Sinusoidal macrophages: brown pigment	1	0	3	3
Bile stasis	0	0	0	0
Sternal marrow				
Hypercellularity	0	0	0	2
Femoral marrow				
Hypercellularity	0	0	0	1
Females				
Liver				
Enlarged (gross lesion)	0	0	4	2
Sinusoidal macrophages: brown pigment	0	0	4	4
Bile stasis	0	0	3	4
Sternal marrow				
Hypercellularity	0	0	0	4
Femoral marrow				
Hypercellularity	0	0	0	4

- **Clinical chemistry and haematology:**
  - **↓ in RBC count in M (↓ 26%, stat signif**) and F (↓ 23%, stat signif)
  - **↓ in Hb in M (↓ 22%, stat signif) and F** (↓ 22%, stat signif)
  - **↓ in haematocrit in M (↓ 18%, stat signif**) and F (↓ 16%, stat signif)
  - ↑ MCV in M (↑11% stat signif) and F (↑8 % stat signif)
  - **↑ reticulocyte count in M (↑131% stat signif)** and F (↑ 82% stat signif)
  - ↑ liver enzyme activities:
    - **ALT: ↑ in M (↑65%)** and F (↑ 20%) – not stat signif
    - **ASAT: ↑ in M (↑24%)** and F (↑ 7%) – not stat signif
    - **ALP: ↑ in M (↑97%)** and F (↑ 98% stat signif)

<u>At 146/160 mkd in M/F:</u>

EAS parameters:

- **Testis weight: ↓ absolute testes weight**
- **Testis histopathology: Bilateral tubular atrophy, decrease thickness of the seminiferous tubules,**

cytoplasmic vacuolation of germinal epithelium • **Epididymis histopathology:** Aspermatogenesis, oligospermia ➔ **See Table above.** • **No measurement on:** ○ Uterus histopathology (with cervix) ○ Thyroid histopathology ○ Ovary histopathology
Systemic toxicity: • **Body weight:** No effect. • **Body weight gain (0-13 weeks):** No effect. • **Food consumption (0-13 weeks):** No effect. • **Liver weight:** ↑ in rel-to-body liver weight in M (↑30% stat signif.) and F (↑42% stat signif.) • **Liver histopathology:** Bile stasis (f); Pigmented sinusoidal macrophages (m&f) ➔ **see Table, above** • **Bone marrow: N**o effect • **Clinical chemistry and haematology: N**o effect

At 3.9/3.7 mkd: no EAS effects

- **12-month (feeding) – ID: 7**

(Doses: 0.99/1.2, 26.9/27.7 and 111.8/93.9 mkd in M/F)

No effects on EAS modalities.

The following parameters have been tested:

Vagina histopathology
Uterus histopathology (with cervix)
Thyroid weight
Thyroid histopathology
Testis weight
Testis histopathology
Prostate histopathology (with seminal vesicles and coagulating glands)
Ovary histopathology
Mammary gland histopathology (female)
Epididymis histopathology

Systemic toxicity:
At 111.8/93.9 mkd (**LOAEL**)**:** Mortality (1/5 dogs at the top dose). **Decreased BWG (↓ 42% wks 0-13 and ↓18% weeks 0-52), RBC parameters and liver toxicity (↑ ALP, ↑ liver weight by 36% and centrilobular hepatocellular hypertrophy).**

NOAEL = 26.9/27.7 mkd

Mechanistic in vivo

Aromatase inhibition (in vivo data)

<table>
<tr><th>Reference</th><th>Treatment duration</th><th>Doses</th><th>Observed Effect</th></tr>
<tr>
<td>Study ID: 21e

Hormonal analysis of male rats fed for 1 year (1-year interim sacrifice of the chronic 2-year rat carcinogenicity study)</td>
<td>1 year</td>
<td>0, 10, 100, 750, 1500 ppm

0.406, 4.06, 30.6, 64.5 mg/kg bw/day (gavage)</td>
<td>
• Decreased serum estradiol levels :
13.8%, 34.5%, 68.4%*, 47%* for 0.406, 4.06, 30.6, 64.5 mkd respectively

• Increased LH and FSH levels at 64.5 mkd:
13.3%* and 34%* respectively

• Increased serum testosterone levels :
65% and 89%* for 30.6, 64.5 mkd respectively

Summary of hormone levels from male rats fed triflusulfuron methyl for 1 year (standard deviation)
<table>
<tr><th>Conc mkd</th><th>Testosterone ng/mL</th><th>Estradiol pg/mL</th><th>Prolactin ng/mL</th><th>LH ng/mL</th><th>FSH ng/mL</th></tr>
<tr><td>0</td><td>0.888 (0.576)</td><td>4.852 (3.609)</td><td>1.741 (1.077)</td><td>0.182 (0.055)</td><td>6.742 (1.532)</td></tr>
<tr><td>0.406</td><td>0.810 (0.677)</td><td>4.183 13.8% (3.420)</td><td>2.766 (4.052)</td><td>0.155 (0.032)</td><td>6.197 (1.261)</td></tr>
<tr><td>4.06</td><td>0.966 (0.957)</td><td>3.179 34.5% (3.443)</td><td>1.831 (1.298)</td><td>0.178 (0.053)</td><td>7.279 (1.744)</td></tr>
<tr><td>30.6</td><td>1.467 65% (0.701)</td><td>1.534* 68.4% (1.874)</td><td>1.362 (1.187)</td><td>0.179 (0.050)</td><td>7.594 (1.515)</td></tr>
<tr><td>64.5</td><td>1.677* 89% (1.535)</td><td>2.574* 47% (2.836)</td><td>1.602 (0.752)</td><td>0.210 13.3% (0.064)</td><td>9.028* 34% (2.109)</td></tr>
</table>
* Statistically significant by Jonckheere's test, p <0.05 (from HLR 575-93, Supplement No. 1)
</td>
</tr>
<tr>
<td>Study ID: 21a

In vivo mechanistic 2-week oral (gavage) study in male rat (HLR 575-</td>
<td></td>
<td></td>
<td>
• Decreased absolute accessory sex glands weight (all doses)
• Decreased serum estradiol levels (all doses)
• Increased LH, FSH and PRL levels (not statistically significant with high inter-individual variations)
• Decreased hepatic aromatase activity (not statistically significant with high inter-individual variation)

All the above mentioned effects were observed in the presence of severely decreased BW and BW gain. However, they were not observed in the same magnitude
</td>
</tr>
</table>

<table>
<tr>
<td>93). Start of dosing in animals 79 weeks old.</td>
<td>2 weeks</td>
<td>0, 1000, 1500, 2000 mg/kg bw/day

+ pair-fed control to 2000 mg/kg bw/day</td>
<td>or were not observed at all in the pair-fed (to 2000 mkd) control (a group with equally decreased BW and BW gain):

Table: Percentage change in comparison to control (ad libitum)
<table>
<tr><th>Param.</th><th>0 mkd (ad lib.)</th><th>0 mkd (pair-fed to 2000 mkd)</th><th>1000 mkd</th><th>1500 mkd</th><th>2000 mkd</th></tr>
<tr><td>BW</td><td>-</td><td>12.7%*</td><td>10.2%*</td><td>14%*</td><td>13.5%*</td></tr>
<tr><td>BWG</td><td>-</td><td>81%*</td><td>73.9%*</td><td>96%*</td><td>87.9%*</td></tr>
<tr><td>Absolute accessory sex glands weight (g)</td><td>-</td><td>↓16.9%*</td><td>↓26%*</td><td>↓34.7%*</td><td>↓29.7%*#</td></tr>
<tr><td>Oestradiol (pg/mL)</td><td>-</td><td>↓14%</td><td>↓68%*</td><td>↓86%*</td><td>↓86.6%*#</td></tr>
<tr><td>LH (ng/mL)</td><td>-</td><td>↑12.3%</td><td>↑33%</td><td>↑13.5%</td><td>↑59.9%</td></tr>
<tr><td>FSH (ng/mL)</td><td>-</td><td>↑2.4%</td><td>↑41%</td><td>↑40.8%</td><td>↑22.2%</td></tr>
<tr><td>PRL (ng/mL)</td><td>-</td><td>↓</td><td>↑28%</td><td>↑37.2%</td><td>↑50.2%</td></tr>
<tr><td>Hepatic aromatase activity (ng/mL)</td><td>-</td><td>↑</td><td>↓17.5%</td><td>↓26%</td><td>↓18.5%</td></tr>
</table>
* Significantly different from ad libitum control
# Significantly different from pair-fed control by the one-way analysis of variance and Dunnett's test criteria, alpha = 0.05

hCG stimulation performed only at 2000mg/kg:
• Increased serum testosterone level in 2000 mg/kg bw/day treated hCG-stimulated rats
• Decrease serum estradiol level in 2000 mg/kg bw/day treated hCG-stimulated rats</td>
</tr>
<tr>
<td>Study ID: 11

Effects of triflusulfuron methyl on hormonal concentrations in male rats</td>
<td>28 days</td>
<td>0, 0.1, 0.5, 5 mg/kg bw/day</td>
<td>Slight effects on serum estradiol levels:

• Mean rate of serum estradiol increase over the dosing period (from pre-study to week 4) was statistically lower in the rats dosed with 5 mg/kg bw/day compared to the control group (decrease of 44%).
• Not statistically significant decrease in estradiol levels (5.3%, 18%, 26% at 0.1, 0.5 and 5 mg/kg bw/day)
• No clinical signs of toxicity, no effects on body weight, food consumption, or food efficiency.</td>
</tr>
</table>

Study ID: 12 In vivo mechanistic 14-day (gavage) in rat	**14 days**	**0, 1500, 2000 mg/kg bw/day**	**Inconclusive results due to small sample size (N=4) and high variability of the results between treated groups:** • No statistically significant increase in the clearance values of [^{14}C]testosterone : mean clearance values CL0–6: 583, 838 and 889 mL/h/kg for the control, 1500 mg/kg bw/day and 2000 mg/kg bw/day dose groups respectively
Study ID: 20 15-day intact male rat assay for detecting endocrine activity	**14 days**	**0, 500, 1000, 1500, 2000 mg/kg bw/day (gavage)**	**Results from the O'Connor study were not reproduced:** • Decreased bw and bwg • Not statistically significant decrease in absolute seminal vesicles weight • No effect on estradiol, testosterol, LH and FSH levels • Statistically significantly decreased estradiol levels only after hCG challenge at 2000 mg/kg bw/day • Statistically significantly increased FSH levels only after hCG challenge at 2000 mg/kg bw/day

Estrogen and Androgen receptor (in vivo data)

- The active substance did not show any agonistic or antagonistic activity for AR in an OECD 458 mechanistic study for concentrations up to 10 µM.
- The active substance did not show any agonistic or antagonistic activity for ER in an OECD 455 mechanistic study for concentrations up to 10 µM.

Mechanistic in vitro

Aromatase activity (in vitro data): Results indicate a weak inhibitory response in aromatase (at high concentrations). An IC50 could be calculated only after C8-induction of aromatase activity (IC50 = 174 µM).

Test system	Concentrations tested	Results	Reference
Hepatic microsomes from C8-treated male rats	**10 – 500 µM**	• Statistically **significant inhibition of C8-induced aromatase activity** at all doses • IC50 = 174 µM • Weak inhibitory response	ID:21d
Hepatic microsomes from male rats (non induced)	**0.1 – 500 µM**	• **Inhibition of aromatase activity** • No IC50 could be calculated (25% inhibition at 500 µM) • Weak inhibitory response • No statistical analysis	ID:17

<table>
<tr>
<td>Isolated Leydig cells</td>
<td>0.1 – 1000 µM</td>
<td>

Statistically significant decreased estradiol levels at the two highest concentrations tested (100 and 1000 µM) in hCG-stimulated and non-stimulated Leydig cells (also non statistical significant decrease at 10 µM).
Statistically significant increase in testosterone production by non-hCG-stimulated Leydig cells at 1000 µM
No effect on the biosynthesis of testosterone by hCG-stimulated Laydig cells

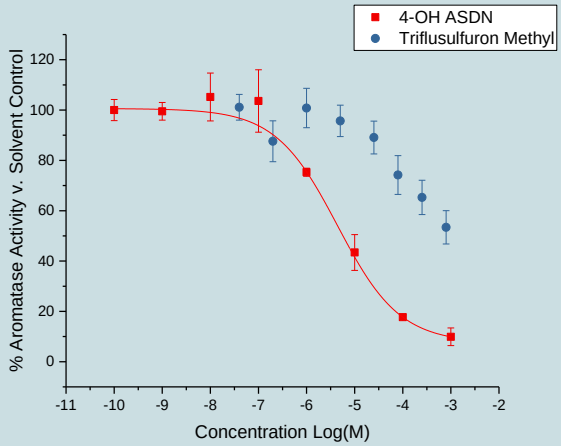
Summary of the effect on isolated Leydig cells
<table>
<tr><th rowspan="2">Tested concentration (µM)</th><th colspan="2">Testosterone (ng/mL)</th><th colspan="2">Oestradiol (pg/mL)</th></tr>
<tr><th>+hCG</th><th>-hCG[a]</th><th>+hCG[a]</th><th>-hCG[a]</th></tr>
<tr><td>0</td><td>14.603
(1.669)</td><td>3.297
(0.091)</td><td>18.739
(3.747)</td><td>9.956
(1.829)</td></tr>
<tr><td>0.1</td><td>15.194
(1.214)</td><td>2.736
(0.246)</td><td>17.262
(0.721)</td><td>8.393
(1.022)</td></tr>
<tr><td>0.5</td><td>14.726
(0.747)</td><td>3.479
(0.944)</td><td>15.795
(2.754)</td><td>9.795
(1.637)</td></tr>
<tr><td>1</td><td>14.611
(0.868)</td><td>4.186
(0.771)</td><td>18.316
(1.309)</td><td>10.895
(1.818)</td></tr>
<tr><td>10</td><td>15.505
(0.398)</td><td>4.838
(0.374)</td><td>15.440
17.6%
(0.323)</td><td>9.613
(1.989)</td></tr>
<tr><td>100</td><td>16.142
(1.574)</td><td>4.585
39.1%
(1.168)</td><td>12.969[b]
30.8%
(2.574)</td><td>4.836[b]
51.4%
(2.693)</td></tr>
<tr><td>1000</td><td>13.250
(2.873)</td><td>6.556[b]
98.9%
(1.628)</td><td>8.113[b]
56.7%
(1.013)</td><td>4.825[b]
51.5%
(1.178)</td></tr>
</table>
[a]Test for linear trend over groups indicates a trend at alpha = 0.05.
[b]Significantly different from ad libitum control by the one-way analysis of variance and Dunnett’s test criteria, alpha = 0.05.
</td>
<td>ID:21f</td>
</tr>
<tr>
<td>Human recombinant CYP19 microsomes</td>
<td>0.0406 – 800 µM</td>
<td>

No inhibition (release of tritiated water from [^{3}H]-androstenedione)

</td>
<td>ID:19</td>
</tr>
<tr>
<td>Hepatic microsomes from male rats (non induced)</td>
<td>0.0406 – 800 µM</td>
<td>

Inhibition of aromatase activity (release of tritiated water from [^{3}H]-androstenedione)
No IC50 was calculated (50% inhibition at 800 µM)
Weak inhibitory response
No statistical analysis

Rat Hepatic Microsomes Aromatase Activity Assay: Test chemical and Positive Control, 4-OH ASDN
</td>
<td>ID:18a</td>
</tr>
</table>



<table>
<tr>
<td>Ovary homogenate from female SD rats</td>
<td>0.0406 – 800 µM</td>
<td>

Inconclusive results

Rat ovary homogenate aromatase activity assay (% estradiol vs solvent control)
<table>
<tr><th rowspan="2">Test substance</th><th rowspan="2">Final concentration (M)</th><th colspan="2">Run 1</th><th colspan="2">Run 2</th></tr>
<tr><th>Mean[a]</th><th>SD[a]</th><th>Mean[a]</th><th>SD[a]</th></tr>
<tr><td>DMSO[b] + Testosterone</td><td>—</td><td>100.0</td><td>8.1</td><td>100.0</td><td>5.9</td></tr>
<tr><td>Buffer[c] + Testosterone</td><td>—</td><td>87.9</td><td>8.2</td><td>102.3</td><td>16.9</td></tr>
<tr><td rowspan="8">4-OH ASDN (positive control)</td><td>1×10^{-10}</td><td>88.6</td><td>9.5</td><td>103.6</td><td>11.2</td></tr>
<tr><td>1×10^{-9}</td><td>101.3</td><td>5.5</td><td>96.0</td><td>25.8</td></tr>
<tr><td>1×10^{-8}</td><td>64.2</td><td>11.3</td><td>83.8</td><td>22.5</td></tr>
<tr><td>$1 \times 10^{-7.5}$</td><td>54.5</td><td>9.0</td><td>57.3</td><td>3.6</td></tr>
<tr><td>1×10^{-7}</td><td>16.8</td><td>3.6</td><td>17.6</td><td>5.6</td></tr>
<tr><td>$1 \times 10^{-6.5}$</td><td>2.0</td><td>1.1 (n=2)</td><td>2.3</td><td>6.2</td></tr>
<tr><td>1×10^{-6}</td><td>-0.6</td><td>5.0</td><td>-12.9</td><td>3.2</td></tr>
<tr><td>1×10^{-5}</td><td>-10.4</td><td>2.0</td><td>-20.8</td><td>1.1</td></tr>
<tr><td rowspan="8">Triflusulfuron methyl</td><td>4.0×10^{-8}</td><td>77.9</td><td>2.3 (n=2)</td><td>94.8</td><td>6.2</td></tr>
<tr><td>2.0×10^{-7}</td><td>83.6</td><td>5.6 (n=2)</td><td>91.3</td><td>9.3</td></tr>
<tr><td>1.0×10^{-6}</td><td>70.6</td><td>13.0</td><td>96.1</td><td>11.6</td></tr>
<tr><td>5.1×10^{-6}</td><td>63.2</td><td>16.2</td><td>98.0</td><td>15.6</td></tr>
<tr><td>2.5×10^{-5}</td><td>73.2</td><td>8.8</td><td>83.4</td><td>17.5</td></tr>
<tr><td>8.0×10^{-5}</td><td>82.2</td><td>25.4</td><td>92.8</td><td>17.8</td></tr>
<tr><td>2.5×10^{-4}</td><td>52.7</td><td>13.6</td><td>97.6</td><td>16.8</td></tr>
<tr><td>8.0×10^{-4}</td><td>53.7</td><td>2.1</td><td>89.6</td><td>13.7</td></tr>
</table>

</td>
<td>ID:18a</td>
</tr>
<tr>
<td>Ovary homogenate from female SD rats</td>
<td>0.0001 – 500 µM</td>
<td>

Dose dependent inhibition of aromatase activity
No IC50 could be calculated (22% and 30% reduction in estradiol concentration at 100 and 500 µM, respectively)
No statistical analysis

</td>
<td>ID:33</td>
</tr>
</table>

<table>
<tr>
<td></td>
<td></td>
<td>
% Estradiol formed at 100 µM and 500 µM triflusulfuron methyl in experiment 1 and 2.
<table>
<tr><th>Test item concentration</th><th>Experiment No.</th><th>% Estradiol formed compared to full activity control</th><th>Average % estradiol formed across experiments</th></tr>
<tr><td rowspan="2">100 µM</td><td>1</td><td>71.0% [79.4%]</td><td rowspan="2">78% [82%]</td></tr>
<tr><td>2</td><td>85.3%</td></tr>
<tr><td rowspan="2">500 µM</td><td>1</td><td>64.0% [71.7%]</td><td rowspan="2">70% [74%]</td></tr>
<tr><td>2</td><td>76.1%</td></tr>
</table>
Values between […]: % Estradiol formed compared to full activity control obtained when excluding replicate C in experiment 1.
</td>
<td></td>
</tr>
<tr>
<td>Human placental cell line (JEG-3)</td>
<td>0.01, 0.04, 0.16, 0.6, 2.5, 10, 40 µM</td>
<td>

↓E2 (70%*) at 40µM
No effect in progesterone
No changes in CYP19 gene expression

</td>
<td>ID:22</td>
</tr>
</table>

Dopamine receptor (in vitro data)

- The active substance did not specifically bind to the dopamine D2 receptor in the in vitro dopamine receptor binding assay up to 1.34 mM.

Table 2. EAS-mediated patterns of adversity (effects on testes and epididymides)

Study ID	Duration	Effect dose (mkd)	Testes weight	Testes histopathology	Epididymides weight	Epididymides histopathology	Hormones/Enzymes	Systemic toxicity
11	28-days	0.1					• ↓ serum estradiol levels (5.3%)	• No clinical signs of toxicity, • No effects on body weight, • No effects on food consumption, or food efficiency.
3	22-month	0.406	No effect	No effect	Not measured	No effect		Dose below NOAEL
11	28-days	0.5					• ↓ serum estradiol levels (18%)	• No clinical signs of toxicity, • No effects on body weight, • No effects on food consumption, or food efficiency.
8	2-gen	0.588	No effect	No gross lesions	Not measured	No gross lesions		Dose below NOAEL
3	22-month	4.06	No effect	No effect	Not measured	No effect		Study NOAEL
11	28-days	5					• ↓ serum estradiol levels (26%) • Lower rate (by 44%)* of estradiol increase with time	• No clinical signs of toxicity, • No effects on body weight, • No effects on food consumption, or food efficiency.
8	2-gen	5.81	No effect	No measured	Not measured	No measured	Not measured	Study NOAEL
2	90-day	6.2	No effect	No effect	Not measured	No effect	Not measured	Study NOAEL
1	90-day	6.6	No effect	Not measured	Not measured	Not measured	Not measured	Study NOAEL
3a, 21e	12-month	**30.6**	**↑ rel. testis weight (14%, not stat. sign)**	No effect	Not measured	No effect	• ↓ serum estradiol levels (68.4%)* • ↑ serum testosterone (65.0%)	• Haematology (anaemia) in M • ↓ BW (7%) in M • ↓ BW gain (11%) in M

Study ID	Duration	Effect dose (mkd)	Testes weight	Testes histopathology	Epididymides weight	Epididymides histopathology	Hormones/Enzymes	Systemic toxicity
3b	22-month	**30.6**	No effect	**↑ Leydig cell hyperplasia (36.0%, stat. sign) and** **↑ Leydig cell adenoma (14%, stat. sign) at terminal sacrifice. Outside HC data.**	Not measured	No effect	Not measured	• Haematology (anaemia) in M • ↓ BW (5%) in M • ↓ BW gain (8%) in M
8	2-gen	**44.0**	**↑ (6%, stat. sign.) in relative testis weight in F0. No effect in abs. testis weight. No effect in F1**.	Not measured	Not measured	Not measured	Not measured	• ↓ food consumption (4%) and food efficiency (9%, stat. sign) in M (F0) • F0: ↓ BW (5%, stat. sign in M) & BW gain (13%, stat. sign. in M)
3a, 21e	12-month	64.5	**↑ rel. testis weight (28%, stat. sign) and ↑ abs. testis weight (6%, not stat. sign) at interim sacrifice.**	No effect	Not measured	No effect	• ↓serum estradiol levels (47%*) • ↑ serum testosterone (89.0%)* • ↑ LH (13.3%*) • ↑ FSH (34%*)	• Haematology (anaemia) in M • ↓ BW (14% in M, 16% in F) • ↓ BW gain (20% in M, 28% in F) • ↑ in rel. liver weight in F • ↑ rel. brain weight in M and F **Above the MTD**
3b	22-month	64.5	**↑ rel. testis weight (23%, stat. sign) and ↑ abs. testis**	↑ Leydig cell hyperplasia stat. sign (52.9% vs	Not measured	No effect	Not measured	• ↓ BW (14% in M, 15% in F) • ↓ BW gain (20% in M, 23% in F) • 54% survival for M and 46% for F. (According to the study author, the poor

Study ID	Duration	Effect dose (mkd)	Testes weight	Testes histopathology	Epididymides weight	Epididymides histopathology	Hormones/Enzymes	Systemic toxicity
			weight (5%, not stat. sign) at terminal sacrifice	19.6% in controls) and ↑ Leydig cell adenoma stat. sign (13.7%, vs 0% in controls)				survival rate is not a treatment related effect but typical for this strain of rat (Crl:CD BR) • ↑ rel. brain weight in M and F • ↓ abs. kidney weight, • ↓ abs. spleen weight (19%, stat. sign) in F **Above the MTD**
8	2-gen	89.5	**↑ relative testis weight in F0 (10%, stat. sign) and F1 (16%, stat. sign.). No effect in abs. testes weight.**	No effect in F0 and F1	Not measured	No effect in F0 and F1	Not measured	• ↓ food consumption • ↓ food efficiency (> 10%) • ↓ BW (10%) & BW gain (22%) in F0/F1 M • ↓ Pup weight (7%, stat. sign) - F1 **Above the MTD**
2	90-day	127	No effect	No effect	Not measured	No effect	Not measured	• Spleen weight ↓ (rel) • Liver weight ↑ (rel) • Kidney weight ↓ (abs) • Kidney histopathology • Food consumption & efficiency ↓ • Clinical chemistry and haematology (anaemia) • Brain weight ↓ (abs) • Body weight ↓ (9% in M, 16% in F)
1	90-day	133	No effect	Not measured	Not measured	Not measured	Not measured	• Body weight ↓ (<10%) • Food efficiency ↓ in M • Spleen histopathology in F • Liver weight ↑ (rel) in F
20	15-Days	500	Not measured	Not measured	Not measured	Not measured	No effect on: • serum estradiol • testosterone	• ↓ Body weight • ↓ Body weight gain • No clinical signs

Study ID	Duration	Effect dose (mkd)	Testes weight	Testes histopathology	Epididymides weight	Epididymides histopathology	Hormones/Enzymes	Systemic toxicity
							• LH • FSH	• **↓ absolute seminal vesicles weight (8.7%)**
2	90-day	**646**	No effect	**Atrophy/degeneration, seminiferous tubules, bilateral**	Not measured	**Oligospermia, bilateral Oligospermia, unilateral Sperm granuloma**	Not measured	• Body weight ↓ (30% in M, 29% in F) • Body weight gain (0-91 days) ↓ • Food consumption (0-91 days) ↓ • Food efficiency (0-91 days) ↓ • Spleen weight ↑ (rel) • Liver weight ↑ (rel) • Kidney weight ↓ (abs) & ↑ (rel) • Kidney histopathology • Clinical chemistry and haematology (anaemia) • Brain weight ↓ (abs) & ↑ (rel) in M **Dose above MTD**
1	90-day	658	**↑ absolute testes weight (↑ 12% stat signif)**	Not measured	Not measured	Not measured	Not measured	• Body weight ↓ (12% in M, 14% in F) • Food consumption ↓ in F • Food efficiency (0-91 days) ↓ • Spleen weight ↑ (rel) • Spleen histopathology • Liver weight ↑ (rel) in F • Kidney histopathology • Heart weight ↑ (rel) • Clinical chemistry and haematology (anaemia) • Brain weight ↑ (rel) **Dose above MTD**
2	90-day	965	**↓ absolute testes weight (↓ 31% stat signif).**	**Atrophy, degeneration, bilateral Leydig cell hyperplasia.**	Not measured	**Oligospermia, atrophy**	Not measured	• Body weight ↓ (40% in M, 35% in F) • Body weight gain (0-91 days) ↓ • Food consumption (0-91 days) ↓ • Food efficiency (0-91 days) ↓

Study ID	Duration	Effect dose (mkd)	Testes weight	Testes histopathology	Epididymides weight	Epididymides histopathology	Hormones/Enzymes	Systemic toxicity
				Gross observations: small testes				• Spleen weight ↑ (rel) • Liver weight ↑ (rel) • Kidney weight ↓ (abs) & ↑ (rel F) • Kidney histopathology • Clinical chemistry and haematology (anaemia) • Brain weight ↓ (abs M) & ↑ (rel) **Dose above MTD**
21a, b, c	15-Days	1000	No effect in abs. testis weight.	Not measured	Not measured	Not measured	• ↓serum estradiol levels (68%*) • ↑ LH (33%) • ↑ FSH (41%) • ↑ PRL (28%) • ↓ Hepatic aromatase (17.5%)	• ↓ Body weight (10%) • ↓Body weight gain (74%) • ↓ **Absolute accessory sex glands (prostate, coagulating glands, seminal vesicles) weight (g) – 26%**
1	90-day	1036	**↑ Absolute testis weight (5% not stat signif)**	No effects	Not measured	No effects	Not measured	• Body weight ↓ (19% in M, 17% in F) • Food consumption ↓ • Food efficiency (0-91 days) ↓ • Spleen weight ↑ (rel) • Spleen histopathology • Liver weight ↑ (rel) • Kidney weight ↑ (rel) • Kidney histopathology • Heart weight ↑ (rel) • Clinical chemistry and haematology (anaemia) • Brain weight ↑ (rel) **Dose above MTD**
21a, b, c	15-Days	1500	Not measured	Not measured	Not measured	Not measured	• ↓serum estradiol	• ↓ Body weight (14%)

Study ID	Duration	Effect dose (mkd)	Testes weight	Testes histopathology	Epididymides weight	Epididymides histopathology	Hormones/Enzymes	Systemic toxicity
							levels (86%*) • ↑ LH (13.5%) • ↑ FSH (40.8%) • ↑ PRL (37.2%) • ↓ Hepatic aromatase (26%)	• ↓Body weight gain (96%) • ↓ **Absolute accessory sex glands (prostate, coagulating glands, seminal vesicles) weight (g) – 34.7%*** • **Dose above MTD**
12	14-Days	1000	Not measured	Not measured	Not measured	Not measured	• ↑ in testosterone clearance (Inconclusive results due to small sample size (N=4) and high variability of the results between treated groups)	
20	15-Days	1000	Not measured	Not measured	Not measured	Not measured	No effect on: • serum estradiol • testosterone • LH • FSH	• ↓ Body weight • ↓ Body weight gain • No clinical signs • **↓ absolute seminal vesicles weight (19.7%*)**
20	15-Days	1500	Not measured	Not measured	Not measured	Not measured	No effect on: • serum estradiol • testosterone • LH • FSH	• ↓ Body weight • ↓ Body weight gain • No clinical signs • **↓ absolute seminal vesicles weight (19.4%*)**
21a, b, c	15-Days	**2000**	Not measured	Not measured	Not measured	Not measured	• **↓serum estradiol levels (86.6%*)** • **↑ LH (59.9%)** • **↑ FSH (22.2%)** • **↑ PRL (50.2%)**	• ↓ Body weight (13.5%) • ↓Body weight gain (87.9%) • ↓ **Absolute accessory sex glands (prostate, coagulating glands, seminal vesicles) weight (g) – 29.7%***

Study ID	Duration	Effect dose (mkd)	Testes weight	Testes histopathology	Epididymides weight	Epididymides histopathology	Hormones/Enzymes	Systemic toxicity
							• **↓ Hepatic aromatase (18.5%)** **Pairfed control animals:** • **↓serum estradiol levels (14%)** • **↑ LH (12.3%)** • **↑ FSH (2.4%)** • **PRL: no increase** • **Hepatic aromatase: no decrease** **With hCG challenge** • **↑Testosterone (92%)** • **↓E2 (75.6%)**	Pairfed control animals: • ↓ Body weight (12.7%) • ↓Body weight gain (81%) • ↓ **Absolute accessory sex glands (prostate, coagulating glands, seminal vesicles) weight (g) – 16.9%** With hCG challenge • ↓ **Absolute accessory sex glands (prostate, coagulating glands, seminal vesicles) weight (g) – 32.5%***
12	14-Days	2000	Not measured	Not measured	Not measured	Not measured	• ↑ in testosterone clearance (Inconclusive results due to small sample size (N=4) and high variability of the results between treated groups)	
20	15-Days	2000	Not measured	Not measured	Not measured	Not measured	No effect on:	• ↓ Body weight

Study ID	Duration	Effect dose (mkd)	Testes weight	Testes histopathology	Epididymides weight	Epididymides histopathology	Hormones/Enzymes	Systemic toxicity
							• serum estradiol • testosterone • LH • FSH Changes after hCG challenge: • ↓serum estradiol levels (22%) • ↑ FSH (23.5%)	• ↓ Body weight gain • No clinical signs • **↓ absolute seminal vesicles weight (10.4%)**

Overall conclusion for EAS-modalities:

Hazard

MOA analysis

Uncertainty analysis