Calculating Estrous Cycle Length Versus Number of Estrous Cycles
A Comparison of Results Based on Nearly 30 Years of Historical Control Data in the Sprague Dawley Rat

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1 Abstract

The rodent estrous cycle is a sensitive indicator of normal reproductive function. Changes to normal cycling patterns are affected by exposure to xenobiotics and chemicals. The Testing Facility routinely evaluates estrous cycles in out-bred rodent strains in a variety of reproductive toxicity study designs, including ICH-compliant fertility, perinatal/postnatal development, and juvenile toxicity studies for pharmaceuticals and OECD-compliant reproductive screens and multigenerational and peripubertal studies for chemicals and agrochemicals.

2 Safety Assessments

In a typical reproductive toxicity assessment for a pharmaceutical candidate, the number of estrous cycles within a designated observation period (e.g., prior to initiation of cohabitation with a male rat of the same source and strain) is determined by counting the number of times estrus (i.e., the period in which cornified epithelial cells are the predominant cell type in a vaginal lavage sample) is observed for each female, averaged as a mean per dose group, and compared to a concurrent control group for statistically significant differences.

3 Endocrine Disruptor Screening

Within the Endocrine Disruptor Screening Program (EDSP) for chemicals, the mean cycle length is determined by counting "the number of days from either one proestrus to the next proestrus, or from one diestrus to the next diestrus."[1] The average cycle length is determined on an individual animal basis, averaged for each dose group, and then compared to a concurrent control group for statistically significant differences.
Estrous evaluations begin on the day that full vaginal patency is achieved and continue through the day of scheduled euthanasia.

[1] Endocrine disruptor screening program test guidelines: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats, OPPTS 890.1450; October 2009; Prevention, Pesticides and Toxic Substances. U.S. Environmental Protection Agency.

Rat No.	Sun	Mon	Tues	Wed	Thurs	Fri	Sat	Sun	Mon	Tues	Wed	Thurs	Fri	Sat	No. Cycles	Mean Cycle Length
1	M	D	P	E	M	D	P	E	M	D	P	E	M	D	3	4
2	E	D	D	P	E	M	D	P	E	M	D	P	E	M	4	4
3	P	E	D	D	E	M	D	D	P	E	M	D	P	E	4	3.5
4	M	E	D	D	P	E	D	D	P	E	M	D	P	E	4	3.5
5	M	P	E	D	D	P	E	M	D	P	E	D	D	P	3	3.5
E = Estrus; M = Metestrus; D = Diestrus; P = Proestrus														Means	3.6	3.7

4 Applicability

The mean cycle length method of evaluating estrous can be applied to both safety assessment designs and endocrine disruptor screening studies.

Rat No.	PND 32	PND 33	PND 34	PND 35	PND 36	PND 37	PND 38	PND 39	PND 40	PND 41	PND 42	Mean Cycle Length	No. Cycles
6							D	D	P	E	P	a	1
7						P	E	D	D	D	P	a	1
8			D	D	D	P	E	D	D	D	E	3	2
9	D	E	D	D	D	D	E	D	D	P	E	2	3
10						D	D	D	P	E	D	3	1
E = Estrus; D = Diestrus; P = Proestrus											Means	2.7	1.6

[a] Mean cycle length could not be calculated.

5 Limitations

Peripubertal Development and Thyroid Function studies within the EDSP screening program are conducted in juvenile rats before sexual maturation is achieved. The dose period within the study is brief, and females are euthanized by Postnatal day 42 (PND 42). Due to the age of the females and the brevity of the study, evaluating the number of estrous cycles does not lend itself to this study design.

- In instances where females do not achieve sexual maturation before scheduled euthanasia, no estrous data may be available.
- In instances of delayed vaginal patency, estrous data may be limited.
- Females may not be cycling (e.g., in persistent diestrus) or cycling irregularly (e.g., more than one instance of diestrus or proestrus may not be present).

The mean cycle length method serves as the best means of evaluating estrous cyclicity using a limited set of data.

6 Historical Control Data

Based on historical control data sets collected since 1992, the background incidence for the number of estrous cycles in the Sprague Dawley (SD) rat has ranged between 3.1 and 3.3 during pre-cohabitation period estrous evaluations.

- Husbandry conditions (e.g., bedding, food, and water) have been essentially the same over this period.
- In late 2014, the Testing Facility implemented a social housing guidance, and therefore, that year is presented individually as a transitional year.
- As the mean number of estrous cycles was the same between 2015 and 2019 as it was prior to 2014, it was considered that social housing versus individual housing had no impact on estrous cyclicity in female SD rats.

Historical Control	1992 – 1999	2000 – 2007	2008 - 2013	2014	2015 – 2019
Mean No. Cycles	3.3 ± 0.265	3.2 ± 0.236	3.2 ± 0.199	3.1 ± 0.416	3.2 ± 0.305

7 Conclusion

Each tabulation technique was applied to the negative control and positive control datasets to compare the sensitivity of each approach in identifying potential effects on estrous in SD female rats.

- The difference between negative and positive controls is apparent by both methods, but the difference is more obvious using the mean number of cycles.
- The mean cycle length method is limited when a specific stage of estrous is persistent, resulting in the exclusion of females from summarization and statistical analysis.
- However, the mean cycle length method also includes an additional qualification of cyclicity (i.e., regular cycling, irregular cycling, or not cycling), which further characterizes these exclusions.

Data Sets	Negative Control (2019 HCD)	Positive Control
No. Studies	8	8
No. of Cycles (Mean ± SD)	3.124 ± 0.440	2.772 ± 0.860
No. of Female Rats	180	177
Mean Cycle Length (Mean ± SD)	3.489 ± 0.543	3.403 ± 0.588
No. of Female Rats	180	165[a]

[a] Excludes 12 females for which mean cycle length could not be calculated.