

# Using *Daphnia magna* Historical Control Data to Evaluate Study Results

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## Abstract

Historical control data (HCD) can be used to support study results when a data set may be unclear due to high or low variability. The reproductive endpoints for *Daphnia magna* chronic testing have changed recently with the finalization of the OCSPP 850.1300 guideline. Additional response variables are now required including F<sub>0</sub> production rate of first brood release and number of live offspring per F<sub>0</sub> adult per reproductive day. Since collection and calculation of these response variables only began after the 850.1300 guideline was finalized in October of 2016, the historical database for these endpoint requirements is limited. Additionally, the statistical evaluation of the multiple reproductive endpoints can lead to conflicting results within a single data set. Since low variability can increase sensitivity of statistical analysis, historical data can assist in such cases to make an argument for adjustment to the statistically determined NOEC value based on these historical data values.

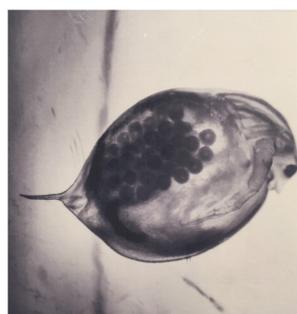


Figure 1. *Daphnia magna*

The recently required daphnid reproduction response variables have low variability in the data sets collected so far; especially the production rate of first brood endpoint. Based on the daphnid life cycle, brood release is typically within a two-day period and with once daily observations, this endpoint does not provide a true biological difference unless outside of these historical ranges. Gathering more HCD on the reproductive endpoints, specifically the production rate of the first brood endpoint, will likely prove to be helpful in instances where low variability yields uncertain or unreasonable endpoint results.

## Historical Control Data

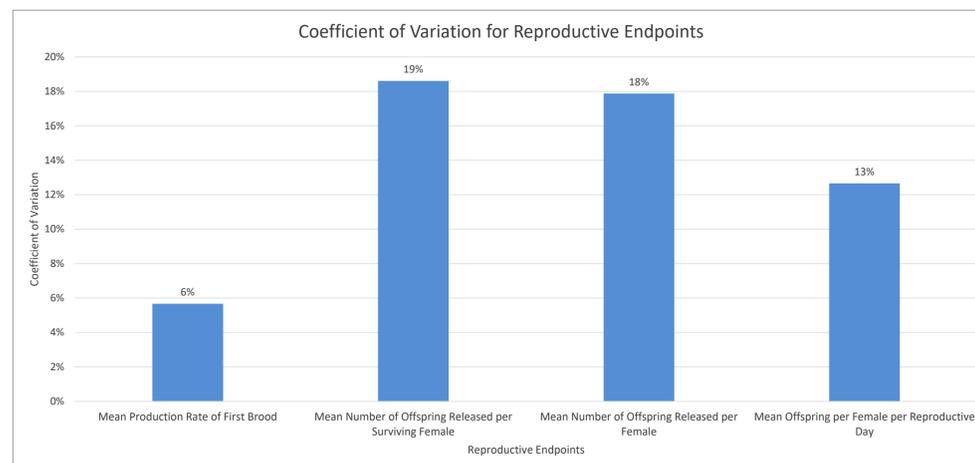
	Mean Production Rate of First Brood	Mean # of Offspring Released per Surviving Female	Mean # of Offspring Released per Female	Mean # of Offspring per Female per Reproductive Day
Min	0.11	109	109	8
Max	0.14	246	214	12
Mean	0.13	162	155	10
Median	0.13	159	157	11

Includes Control and Solvent Control data

N = 73 for Mean # of Offspring Released per Surviving Female

N = 32 for Mean # of Offspring Released per Female.

N = 14 for Mean # of Offspring per Female per Reproductive Day and Mean Production Rate of First Brood.



## Example Data Set 1

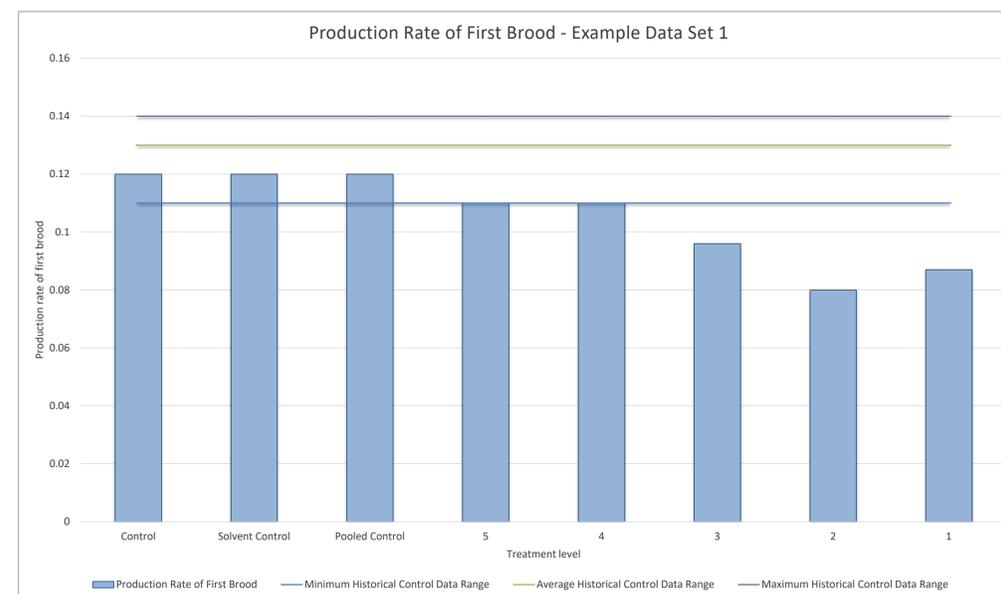
Treatment Group	Day of First Brood Release (days)	Production Rate of First Brood			Offspring per Surviving Female (# offsp.)			Offspring per Female (# offsp.)			Offspring per Female per Reproductive Day (# offsp.)			Mean Percent Survival
		Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	
Control	9	0.12	0.0	0	130	9.7	8	117	42	36	10	1.0	10	80
Solvent Control	9	0.12	0.0071	6	130	5.8	4	117	40	34	10	0.42	4	90
Pooled Control	9	0.12	0.0051	4	130	7.6	6	117	40	34	10	0.75	8	85
5	9	0.11	0.01	10	132	8.4	6	132	8.4	6	10	0.65	6	100
4	9	0.11	0.01	9	120	15	13	120	15	13	9.2	1.2	13	100
3	9	0.096	0.012	13	124	23	19	124	23	19	9.6	1.8	19	100
2	12	0.080	0.0099	12	56	45	80	35	43	123	2.9	3.3	114	60
1	12	0.087	0.0	0	NA	NA	NA	3	6.2	215	0.23	0.51	217	0

SD - Standard Deviation, CV - Coefficient of Variation, NA = Not Applicable (no surviving females present at test termination)

5 = Low treatment level; 1 = high treatment level

Red = Statistically significant

- Statistical analysis for Production Rate of First Brood compared to the pooled control yielded significant effects for all treatment levels.
- Evaluation: a) Production Rate of First Brood for the 5 and 4 treatment levels had a mean of 0.11, (within the HCD range for production rate of first brood). b) These treatment levels only had a 4% effect compared to the pooled control. c) Higher treatment levels (3 and 2) yielded brood production rates of 0.099 and 0.082, respectively, outside of the HCD range and with greater percent effects of 17 and 31%, respectively, compared to the pooled control. d) What does this slight different in time to first brood release mean for a population as opposed to an individual?
- Based on the HCD and the low percent effects in the 5 and 4 treatment levels, the NOEC and LOEC for Production Rate of First Brood were reported as 4 and 3, respectively.



## Example Data Set 2

Treatment Group	Day of First Brood Release (days)	Production Rate of First Brood			Offspring per Surviving Female (# offsp.)			Offspring per Female (# offsp.)			Offspring per Female per Reproductive Day (# offsp.)			Mean Percent Survival
		Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	
Control	8	0.12	0.013	11	171	21	12	154	57	37	11	4.1	37	90
Solvent Control	8	0.13	0.0	0	165	13	8	165	13	20	12	0.95	8	100
Pooled Control	8	0.13	0.01	8	168	17	10	159	41	26	11	2.9	26	95
5	9	0.12	0.0	0	153	18	12	153	18	12	11	1.3	12	100
4	8	0.12	0.013	11	157	8.3	5	157	8.3	5.2	11	0.59	5	100
3	8	0.13	0.012	10	147	11	7	147	11	7.5	10	0.97	9	100
2	9	0.10	0.0092	9	104	21	21	93	38	41	6.7	2.7	41	90
1	12	0.077	0.0059	8	51	16	30	46	22	348	3.7	1.1	30	90

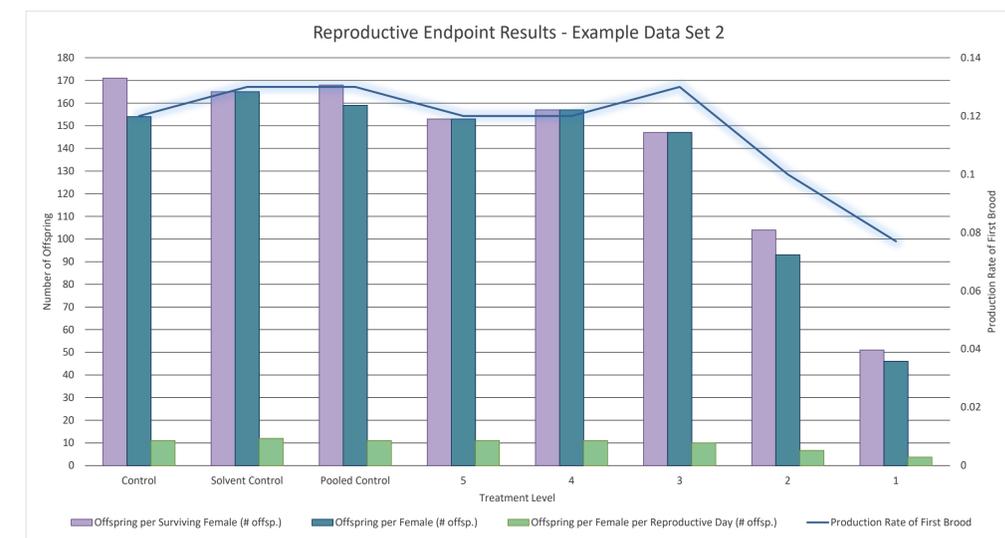
SD - Standard Deviation, CV - Coefficient of Variation

5 = Low treatment level; 1 = high treatment level

Red = Statistically significant

<sup>a</sup> Endpoint not statistically analyzed since no significant reduction in survival was detected.

- Offspring per Surviving Female and Production Rate of First Brood: treatment level 5 compared to the pooled control yielded a statistically significant effect. The percent effect in treatment level 5 was 0.3% above the PMSD (percent minimum significant different) for each endpoint and the next higher treatment level(s) were equivalent to the pooled control.
- Production Rate of First Brood: treatment level 5 was determined to have a significant effect though the mean is 0.12, the same mean value as the control and the next higher treatment level. HCD can be used to scientifically justify that this significant effect is not relevant since the value in the low treatment level is within the HCD range in conjunction with higher levels not determined to be significant.
- The NOEC and LOEC for Production Rate of First Brood were reported as 3 and 2, respectively.
- The NOEC and LOEC for reproduction based on Offspring per Surviving Female were reported as 4 and 3, respectively.



## Conclusions

In both data sets, the lack of variability in the Production Rate endpoint increases the sensitivity of the analysis and yielded statistically significant effects that are within HCD ranges or do not follow a dose response trend. Therefore, evaluation of HCD and consideration of population relevant effects are important in determining reported endpoints.

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