C. elegans adult activity assay: Model for comparative toxicology

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Abstract

We introduce the 16-hour worm Adult Activity Test (wAAT), which can be used to measure *C. elegans* spontaneous locomotor activity levels in order to efficiently screen pharmacological and toxicity induced effects.

A de-novo parametric mathematical model for adult *C. elegans* activity and an example of its application in ranking exposure toxicity are presented.

Introduction

The worm Adult Activity Test (wAAT) is a semi-automated 16-hour assessment of *C. elegans* spontaneous locomotor activity (SLA) in response to oral exposures to chemicals dissolved or suspended in nutrient media.

Data from the wAAT can be used for toxicity screening using a de-novo mathematical model. Model-estimated parameters can be used directly for ranking toxic and pharmacological effects.

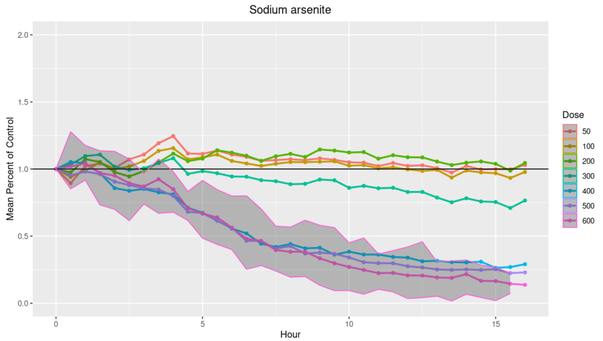

Figure 1. Mean wAAT SLA values for $NaAsO_2$. Mean values are shown in 30-minute increments, standardized first to baseline and then to control. Exposures are in µg/mL. Shaded area is 95% confidence interval around the highest dose, 600µg/mL.

Materials and Methods

PhylumTech wMicroTrackers™ (wMT) uses infrared microbeam interruptions as a measure of within-well, small animal population SLA. The worm Adult Activity Test (wAAT) is a 16-hour assessment of adult population activity levels in multiwell plates. Synchronized adult worms in *C. elegans* habitation medium were exposed to toxic element chemicals and a de novo mathematical model was used to analyze the activity, standardized to control and time zero, over the 16-hour study.

The model-based approach accomplishes two important tasks:

1) it effectively captures the underlying data trend by allocating the noisy part of the data to the model residual error, akin to data smoothing
2) it estimates multiple parametric endpoints which can be used to rank the effects of chemicals and their exposure levels on SLA.

Parametric endpoints used to rank doses into no/low effect, medium effect and high effect include

- peak height (P)
- length of hyperactivity period (t_H)
- activity depression (d).

These methods are applied to a test compound, sodium arsenite (Figures 1 & 2).

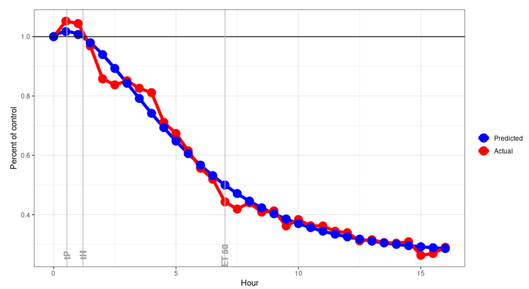

Figure 2. Sodium arsenite observed (mean) values (red) vs. model-predicted values (blue) for the lowest high-effect dose, 400 µg/mL.

Results and Discussion

Sodium arsenite is an example of a hypoactivity-inducing compound with significant hypoactivity induced at higher doses. Model-estimated minimum activity, d, proved effective at ranking doses (Table 1).

This same approach can be used to profile hyperactivity-inducing compounds. The model-estimated endpoints, P and t_H, were effective in distinguishing stimulants, such as caffeine, which express longer hyperactivity phases, from neurotoxicants, such as, mercury chloride, which present escape responses and thereby have shorter hyperactivity phases (Figure 3, Table 2).

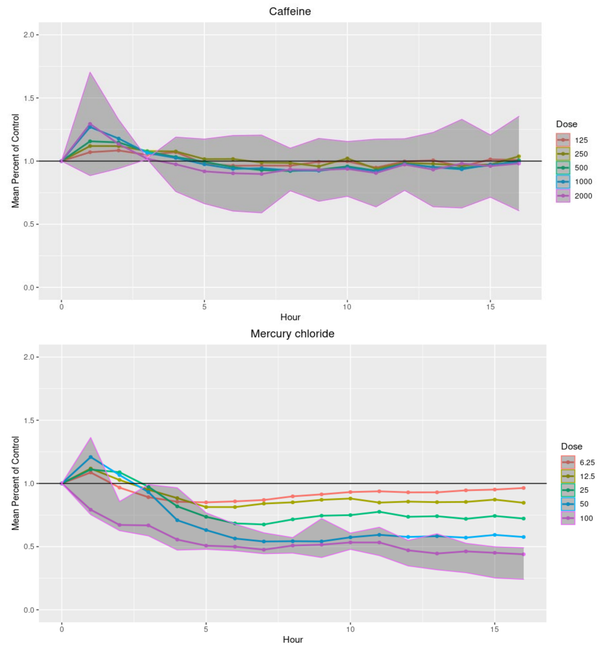

Figure 3. Mean wAAT SLA values for caffeine (top) and mercury chloride (bottom) Shaded area is 95% confidence interval around the highest dose.

Table 1. Assay parameters for sodium arsenite. No/low effect **(green)**. Medium effect **(gold)**. High effect **(red)**.

		Peak activity P		End of hyperactivity phase (hr) tH		Minimum activity d	
compound	dose	Value (95% CI)	Effect Size	Value (95% CI)	Effect Size	Value (95% CI)	Effect Size
NaAsO2	50	1.11 (0.99 , 1.23)	-4.22	14.6 (0 , 29.74)	-20.00	0.91 (0.72 , 1.1)	4.18
	100	1.08 (0.93 , 1.23)	-5.08	12.4 (0 , 30.39)	-20.00	0.76 (0.37 , 1.15)	5.55
	200	1.07 (1.07 , 1.07)	-3.07	6.5 (0 , 321.08)	-20.00	1.07 (1.05 , 1.09)	3.91
	300	1.04 (0.92 , 1.16)	-5.22	4.8 (0 , 10.52)	-20.00	0.66 (0.47 , 0.85)	-2.66
	400	1.02 (0.95 , 1.09)	-4.79	1.2 (0 , 2.42)	-20.00	0.27 (0.22 , 0.32)	-17.17
	500	1.01 (0.96 , 1.06)	-4.65	1 (0 , 2.04)	-20.00	0.2 (0.15 , 0.25)	-19.09
	600	1.04 (0.97 , 1.11)	-4.50	1.7 (0.75 , 2.65)	-12.50	0.1 (0.04 , 0.16)	-21.56

Table 2. Peak parameters for caffeine and mercury chloride.

		Peak activity P	End of hyperactivity phase (hr) tH
compound	dose	Value (95% CI)	Value (95% CI)
Caff	125	1.09 (1.02 , 1.16)	5.4 (3.1 , 7.7)
	250	1.13 (1.05 , 1.21)	6.7 (4.17 , 9.23)
	500	1.17 (1.09 , 1.25)	4.6 (3.57 , 5.63)
	1000	1.27 (1.13 , 1.41)	4.2 (3.15 , 5.25)
	2000	1.32 (1.09 , 1.55)	3.3 (2.22 , 4.38)
HgCl2	6.25	1.19 (0.91 , 1.47)	1.5 (0.67 , 2.33)
	12.5	1.14 (1.04 , 1.24)	2.2 (1.59 , 2.81)
	25	1.16 (1.05 , 1.27)	2.3 (1.73 , 2.87)
	50	1.24 (0.64 , 1.84)	2.3 (1.91 , 2.69)
	100	N.A.*	N.A.*

A web-tool, currently in testing phase, allows anyone to apply the methods described in this poster to their own data

Conclusion

Spontaneous locomotor activity assessment can provide a rapid evaluation of relative acute chemical hazard. The use of the wAAT has the potential to reduce the time and expense of toxicity screening while reducing the use of mammals for further toxicity testing.

The mathematical model presented here is handy in uncovering the underlying trend in activity while providing parametric estimates of endpoints used to rank doses effects and characterize chemical profiles.