

Article

Voluntary Oral Ingestion of a Sedative Prior to Euthanasia with CO₂: Behavioural Responses of Mice

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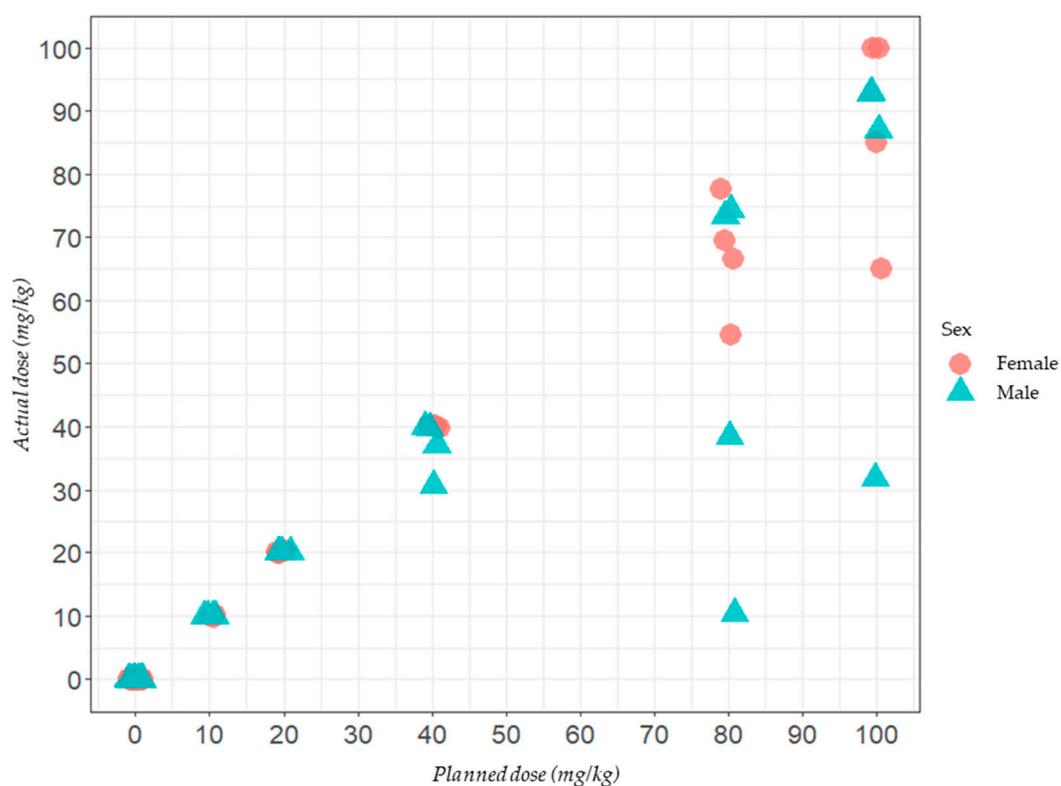


Figure S1. Planned *vs.* actual doses of tiletamine-zolazepam for each individual C57BL/6 male and female mouse. Planned doses were 0 (Control), 10 (Z10), 20 (Z20), 40 (Z40), 80 (Z80) and 100 (Z100) mg/kg, however, not all mice consumed the full amount of dispensed cream cheese on the day of euthanasia. Symbols have been jittered.

Table S1. Continued.

Behaviour	Treatment	Mean	Min	Max	Q1	Median	Q3	SD	n ²
<i>Recumbency⁴ (s)</i>									
	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.00	38
	Z10	0.3	0.0	2.5	0.0	0.0	0.0	0.88	8
	Z20	8.9	0.0	64.6	0.0	0.0	1.6	22.63	8
	Z40	1.8	0.0	9.6	0.0	0.0	1.8	3.39	8
	Z80	342.9	0.0	786.4	0.0	308.4	627.8	357.72	8
	Z100	83.5	1.3	170.5	1.8	81.2	162.9	94.63	4
<i>Walking on lid⁴ (s)</i>									
	Control	87.3	0.0	252.5	47.0	71.0	111.4	63.75	38
	Z10	24.9	0.0	47.4	11.3	24.2	39.6	17.82	8
	Z20	18.7	0.0	82.1	0.0	3.4	24.6	29.52	8
	Z40	27.4	0.0	135.3	0.0	6.7	30.3	46.51	8
	Z80	2.3	0.0	11.1	0.0	0.0	1.9	4.41	8
	Z100	0.0	0.0	0.0	0.0	0.0	0.0	0.00	4
<i>Paddling⁴ (s)</i>									
	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.00	38
	Z10	0.0	0.0	0.0	0.0	0.0	0.0	0.00	8
	Z20	0.0	0.0	0.0	0.0	0.0	0.0	0.00	8
	Z40	0.0	0.0	0.0	0.0	0.0	0.0	0.00	8
	Z80	23.8	0.0	169.7	0.0	0.0	7.4	59.22	8
	Z100	0.0	0.0	0.0	0.0	0.0	0.0	0.00	4
<i>Shaking⁴ (s)</i>									
	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.00	38
	Z10	7.8	0.0	62.7	0.0	0.0	0.0	22.17	8
	Z20	2.4	0.0	19.3	0.0	0.0	0.0	6.82	8
	Z40	1.9	0.0	11.9	0.0	0.0	0.9	4.22	8
	Z80	358.4	0.0	786.4	0.0	284.7	755.3	378.20	8
	Z100	14.0	0.0	43.9	0.0	6.0	20.0	20.74	4

¹ These behaviours were recorded for the first 800 seconds of the observation period.

² Each treatment contained 2 cages of female pair-housed mice and 2 cages of male pair-housed mice; each of these was matched with a randomly assigned control, resulting in a total of 8 control mice per treatment, and a total of 40 control mice for the entire study. There were 2 mice from Control group and 4 mice from Z100 treatment that could not be considered for the behavioural analysis during the sedation period due to a camera recording issue.

³ Jumping and rearing were reported as number of incidents (*frequency, f*).

⁴ Ataxia, immobility, laboured breathing, recumbency, walking on lid, paddling, and shaking were reported as durations (*seconds, s*).

Table S2. Descriptive data for the duration¹ of behaviours recorded during the euthanasia period in C57BL/6 mice

Behaviour	Treatment	Mean	Min	Max	Q1	Median	Q3	SD	n ²
<i>Latency to ataxia from CO₂ started (s)</i>									
	Control	16.0	10.5	20.8	14.7	16.2	17.2	2.36	40
	Z10	16.4	11.4	20.4	15.5	16.1	17.4	2.86	8
	Z20	16.6	13.3	18.7	16.2	16.6	17.3	1.68	8
	Z40	10.1	2.0	16.2	5.7	11.4	15.2	6.11	8
	Z80	11.4	8.3	14.5	9.9	11.3	12.7	2.61	4
	Z100	6.7	2.3	14.0	2.8	3.8	11.1	5.50	6
<i>Latency to recumbency from ataxia (s)</i>									
	Control	8.7	3.8	19.6	6.8	7.9	9.6	3.30	40
	Z10	6.9	4.9	10.5	5.6	6.2	7.4	2.19	8
	Z20	9.5	5.7	14.1	8.5	9.2	10.5	2.45	8
	Z40	14.8	9.2	19.9	11.8	15.0	18.3	4.19	8
	Z80	11.0	9.1	14.4	9.5	10.3	11.8	2.37	4
	Z100	14.6	4.5	20.9	9.5	17.4	19.7	7.07	6
<i>Latency to laboured breathing from recumbency (s)³</i>									
	Control	1.3	-0.5	4.2	0.7	0.9	1.6	0.92	40
	Z10	1.5	0.8	2.4	1.2	1.3	1.8	0.50	8
	Z20	3.1	0.6	8.6	1.8	2.7	3.5	2.46	8
	Z40	1.1	0.3	2.6	0.8	0.9	1.3	0.68	8
	Z80	0.5	-0.9	1.0	0.4	0.9	0.9	0.91	4
	Z100	1.8	0.6	3.0	0.9	1.7	2.7	1.06	6
<i>Latency to last breath from laboured breathing (s)</i>									
	Control	70.4	59.7	99.5	65.7	70.7	73.3	7.33	40
	Z10	71.1	57.7	86.9	62.3	71.6	78.3	10.70	8
	Z20	76.5	62.6	92.5	69.5	75.1	82.8	10.68	8
	Z40	80.8	72.6	100.1	76.0	79.0	82.3	8.78	8
	Z80	83.6	69.6	89.1	82.4	87.8	89.0	9.39	4
	Z100	91.8	83.0	106.2	84.0	90.4	96.8	9.43	6

¹ All behaviours reported as durations (*seconds, s*).

² Each treatment contained 2 cages of female pair-housed mice and 2 cages of male pair-housed mice; each of these was matched with a randomly assigned control, resulting in a total of 8 control mice per treatment, and a total of 40 control mice for the entire study. There were 4 mice in Z80 group and 2 mice in Z100 group that were already recumbent before the euthanasia and could not be considered for the progression of events during the euthanasia.

³ Some mice had already become recumbent prior to experiencing laboured breathing, which resulted in negative values for this variable.