



Article

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

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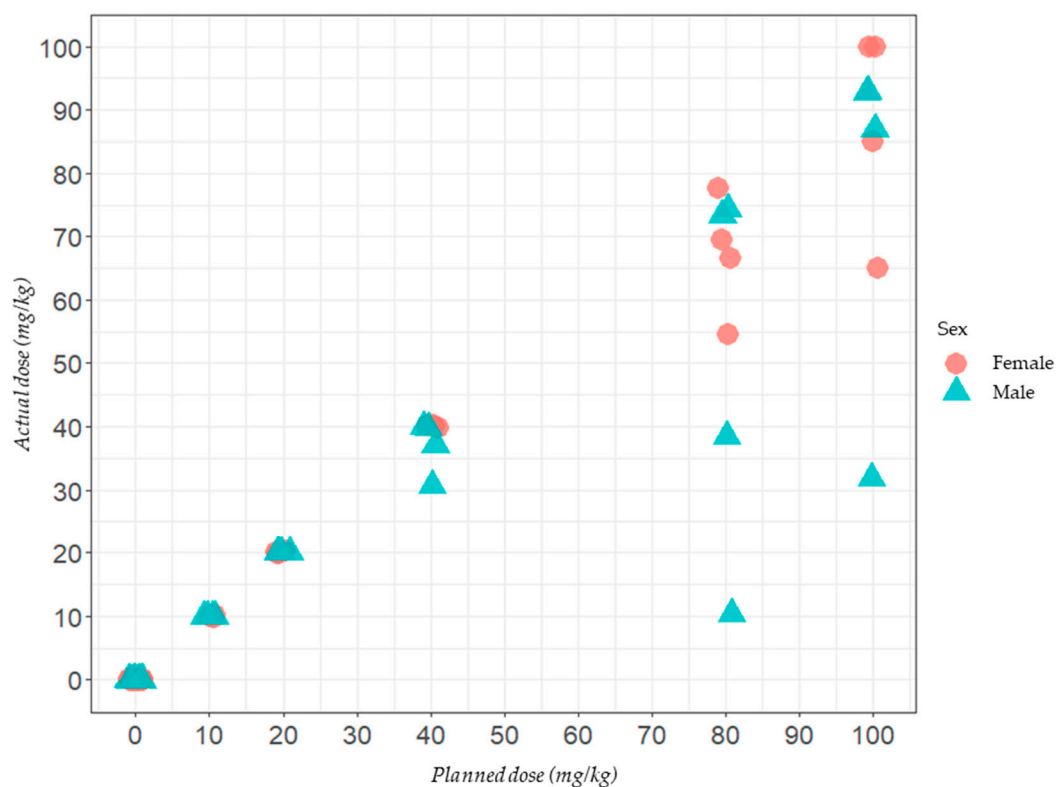


Figure S1. Planned *vs.* actual doses of tiletamine-zolazepam for each individual C57BL/6 male and female mouse. Planned doses were 0 (Control), 10 (Z10), 20 (Z20), 40 (Z40), 80 (Z80) and 100 (Z100) mg/kg, however, not all mice consumed the full amount of dispensed cream cheese on the day of euthanasia.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

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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.