

Table 1. Toxicity study based on the Irwin test in HDHA and DHA treated mice at high doses.

	CONTROL	HDHA		DHA	
		200 mg/kg	500 mg/kg	200 mg/kg	500 mg/kg
Physical factors and gross appearance					
Body weight (g)	28.4 ± 1.3	26.1 ± 1.2	29.4 ± 1.9	28.2 ± 0.9	29.5 ± 2.6
Presence of whiskers	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0
Appearance of fur	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0
Piloerection	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Patches of missing fur on face	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Patches of missing fur on body	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Wounds	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Observation of behavior in a novel environment					
Transfer behavior	4.9 ± 0.1	4.2 ± 0.4	3.3 ± 0.6	4.8 ± 0.3	4.5 ± 0.5
Body position	3.5 ± 0.2	3.2 ± 0.1	3.3 ± 0.3	3.3 ± 0.1	3 ± 0
Spontaneous activity	2.5 ± 0.1	2.4 ± 0.4	1.8 ± 0.5	2.8 ± 0.3	3 ± 0
Respiration rate	2 ± 0	1.8 ± 0.2	2.1 ± 0.1	2 ± 0	2 ± 0
Tremor	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Palpebral closure	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Piloerection	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Gait	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Pelvic elevation	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0
Tail elevation	1.2 ± 0.2	1.4 ± 0.2	1.3 ± 0.3	1.4 ± 0.2	1 ± 0
Urination	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Defecation	1 ± 0.2	1.6 ± 0.6	2.7 ± 1.6	1.5 ± 0.3	2 ± 0
Reflexes and reactions to simple stimuli					
Touch escape	3 ± 0	3 ± 0	2.5 ± 0.3	2.3 ± 0.3	2.5 ± 0.5
Positional passivity	0 ± 0	0 ± 0	0.5 ± 0.3	0 ± 0	0 ± 0
Trunk curl	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Reaching reflex	2.7 ± 0.2	3 ± 0	3 ± 0	3 ± 0	3 ± 0
Body tone	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Measures recorded during supine restraint					
Plantar surface skin color	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Heart Rate	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Limb Tone	1.9 ± 0.1	2.8 ± 0.2	1.8 ± 0.3	2.3 ± 0.3	2 ± 0
Abdominal Tone	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Righting Reflex	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Air Righting Reflex	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Salivation	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Provoked Biting	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Motor coordination					
Grip strength	2.9 ± 0.1	2.8 ± 0.2	1.5 ± 0.3	2.3 ± 0.3	2.5 ± 0.5
Inverted screen (s)	60 ± 0	60 ± 0	52 ± 4.0	60 ± 0	60 ± 0

The Irwin test was assessed as previously described (see reference [67]) according to the attached procedure table (see Online resource 2, Table 2). Animal treatments were performed in 3-months-old WT male mice (n=3 each) as previously described (see reference [40,41]). The Irwin test was evaluated first after an acute dose of 200 or 500 mg/kg of HDHA and DHA (or vehicle, controls), without observing any toxicity evidences. According to that, the treatments were maintained daily for 14 days and the Irwin test was again evaluated at the end of treatment without observing any remarkable physiological alterations.

Table 2. Irwin test parameters and scores.

Physical factors and gross appearance	
Body weight	Animal weight (g) before the test
Presence of whiskers	0 = None; 1 = A few; 2 = Most, but not a full set; 3 = A full set
Appearance of fur	0 = Ungroomed and disheveled; 1 = Somewhat disheveled; 2 = Well-groomed (normal)
Piloerection	0 = None; 1 = Most hairs standing on end
Patches of missing fur on face	0 = None; 1 = Some; 2 = Extensive
Patches of missing fur on body	0 = None; 1 = Some; 2 = Extensive
Wounds	0 = None; 1 = Signs of previous wounding; 2 = Slight wounds present; 3 = Moderate wounds present; 4 = Extensive wounds present
Observation of behavior in a novel environment	
Transfer behavior	0 = Coma; 1 = Prolonged freeze (>10 sec.), then slight movement; 2 = Extended freeze, then moderate movement; 3 = Brief freeze (a few seconds), then active movement; 4 = Momentary freeze, then swift movement; 5 = No freeze, immediate movement; 6 = Extremely excited ("manic")
Body position	0 = Completely flat (on stomach); 1 = Lying on side; 2 = Lying on back; 3 = Sitting or standing; 4 = Rearing on hind legs; 5 = Repeated vertical leaping
Spontaneous activity	0 = None, resting; 1 = Casual scratch, groom, slow movement; 2 = Vigorous scratch, groom, moderate movement; 3 = Vigorous, rapid/dart movement; 4 = Extremely vigorous, rapid/dart movement
Respiration rate	0 = Gasping, irregular; 1 = Slow, shallow; 2 = Normal; 3 = Hyperventilation
Tremor	0 = None; 1 = Mild; 2 = Marked
Palpebral closure	0 = Eyes wide open; 1 = Eyes 1/2 closed; 2 = Eyes closed
Piloerection	0 = None; 1 = Coat stood on end
Gait	0 = Normal; 1 = Fluid but abnormal; 2 = Limited movement only; 3 = Incapacity
Pelvic elevation	0 = Markedly flattened; 1 = Barely touches; 2 = Normal (3mm elevation); 3 = Elevated (more than 3 mm elevation)
Tail elevation	0 = Dragging; 1 = Horizontally extended; 2 = Elevated (Straub tail)
Urination	0 = None; 1 = Little; 2 = Moderate amount; 3 = Extensive
Defecation	Count the number of fecal boli emitted during the 3-min. period
Reflexes and reactions to simple stimuli	
Touch escape	0 = No response; 1 = Mild (escape response to firm stroke); 2 = Moderate (rapid response to light stroke); 3 = Vigorous (escape response to approach)
Positional passivity	0 = Struggles when restrained by tail; 1 = Struggles when restrained by neck (finger grip, not scruffed); 2 = Struggles when held supine (on back); 3 = Struggles when restrained by hind legs; 4 = Does not struggle
Trunk curl	0 = Absent; 1 = Present
Reaching reflex	0 = None; 1 = Upon nose contact; 2 = Upon vibrassee contact; 3 = Before vibrassee contact (18mm); 4 = Early vigorous extension (25mm)
Body tone	0 = Flaccid, no return of cavity to normal; 1 = Slight resistance; 2 = Extreme resistance, board like
Measures recorded during supine restraint	
Plantar surface skin color	0 = Blanched; 1 = Pink; 2 = Bright, deep red flush
Heart Rate	0 = Slow, bradycardia; 1 = Normal; 2 = Fast, tachycardia
Limb Tone	0 = No resistance; 1 = Slight resistance; 2 = Moderate resistance; 3 = Marked resistance; 4 = Extreme resistance
Abdominal Tone	0 = Flaccid, no return of cavity to normal; 1 = Slight resistance; 2 = Extreme resistance, board like
Righting Reflex	0 = No impairment; 1-10 = Number of seconds required to right
Air Righting Reflex	0 = No impairment; 1-10 = Number of seconds required to right
Salivation	0 = None; 1 = Slight margin of sub-maxillary area; 2 = Wet zone entire sub-maxillary area
Provoked Biting	0 = Absent; 1 = Present
Motor coordination	
Grip strength	0 = None; 1 = Slight grip, semi-effective; 2 = Moderate grip, effective; 3 = Active grip, effective; 4 = Unusually effective
Inverted screen (s)	0-60 = Number of seconds before falling