

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 scuffed); 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 |