



Data Article

Analysis of the potential behavioral impact of methanol when used as a solvent: Dataset from zebrafish (*Danio rerio*) behavioral research



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ABSTRACT

Toxicants are commonly administered to experimental organisms using solvents as vehicles. One common vehicle for dissolving toxicants is methanol (CH₃OH), a solvent which on its own is capable of altering physiology and behavior high concentrations. This dataset describes behavioral results in zebrafish (*Danio rerio*) individually exposed to methanol (0.25%, 2.5% vol/vol), or control water, for 30 min prior to behavioral testing. Zebrafish were placed into an open field arena to examine locomotion and zone preference, which was recorded and quantified with motion-tracking software (EthoVision XT). Time spent in the outer (“thigmotaxis”) zone of the arena is a proxy for increased anxiety-like behavior in zebrafish. Additionally, a novel object was placed into the center of the arena to quantify relative increases in boldness/exploration between the methanol and control groups. There were no differences in time spent in any zone of the

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arena or distance travelled between either group, in either test.

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Specifications Table

Subject	Neuroscience: behavioral
Specific subject area	Environmental Science: Health, Toxicology and Mutagenesis Toxicological studies using vehicles/solvents to deliver the toxin of interest may have effects on their own. Here we studied methanol, which is often used to prepare stock solutions, and examined its impact on zebrafish behavior.
Type of data	Figures
How data were acquired	Instruments: Videos were recorded with a Basler monochrome camera with Gigabit Ethernet interface. 4–8 mm F1.4 manual iris and focus lens. The camera was mounted 1 m above the arena with a mounting rod and superclamp. EthoVision XT (version 10, Noldus, VA, USA) was used to record and quantify videos.
Data format	Raw data (accessible on Dryad). Analysed data also added to supplemental (analysed with EthoVision XT).
Parameters for data collection	Data were collected between 9 am and 5 pm in a well-lit temperature-controlled room.
Description of data collection	Raw video files are accessible on Dryad (see excel file on Dryad which describes each video). These data were collected using the motion-tracking software, EthoVision XT. Furthermore, we analysed the time each fish spent within three virtually drawn zones in the arena. The thigmotaxis, transition and center zones were created in EthoVision XT. The total distance each fish travelled during the trial was also collected. These analysed data are accessible in the supplemental materials.
Data source location	Institution: MacEwan University City/Town/Region: Edmonton/Alberta Country: Canada
Data accessibility	With the article as a supplementary file and in the Dryad repository datadryad.org/stash/share/FSKEBkOw8hMLXyjl5NQBj65Z7DIL51pjfviXpSiSqE
Related research article	Hamilton T.J., Krook J., Szaszkievicz J., Burggren. W. Shoaling, Boldness, Anxiety-like Behavior and Locomotion in Zebrafish (<i>Danio rerio</i>) Are Altered by Acute Benzo[A]Pyrene Exposure, <i>Sci. Total Environ.</i> 774 (2021) 145702, https://doi.org/10.1016/j.scitotenv.2021.145702 .

Value of the Data

- This dataset shows that acute exposure to methanol (0.25% or 2.5%) for 30 min does not impact behavior of zebrafish in the open field and novel object approach tests
- The use of methanol as a vehicle for toxicant delivery is common for toxicologists, and at the concentration used here there is no significant impact on behavior.
- Researchers using compounds that require delivery in methanol can consider 0.25% and 2.5% to have little to no effect on behavior.
- It is important to compare vehicles used to deliver toxicants because they may have behavioral or other effects on their own.

1. Data Description

Methanol can act as an ecotoxicant with potential to harm many organisms [1,2]. Our main use of methanol was for its properties as a solvent for B[a]P and was chosen primarily because

there are few effective solvents for this polycyclic aromatic hydrocarbon. Dimethyl sulfoxide (DMSO) is commonly used to dissolve compounds, however, DMSO can alter locomotion in zebrafish [3] and can have sub-lethal effects on larval zebrafish behavior whereas methanol does not at the concentrations studied [4]. Methanol may have sub-lethal behavioral effects on its own in adult zebrafish and warrants careful comparison to control groups. If methanol on its own has effects this can result in complicated interpretations of the primary compound's effects. These experiments also highlight the importance of comparing vehicles used to deliver toxicants to controls. This dataset indicates that methanol alone has no effects on behavior at 0.25% or 2.5%, however, higher doses and other behavioral tests should also be evaluated.

Fig. 1 compares the behavioral impact of methanol (0.25% and 2.5%) to that of controls in the open field test. We observed no effect of methanol on time spent in the thigmotaxis zone (Fig. 1A, $P = 0.224$). There was no effect of methanol on time spent in the transition zone (Fig. 1B, $P = 0.285$). There was no effect of methanol on time spent in the center zone (Fig. 1C, $P = 0.276$). There was no effect of methanol on distance the fish moved (Fig. 1D, $P = 0.2122$). Data in Fig. 1 are presented as means \pm 1 SEM. Control ($n = 25$), 0.25% ($n = 25$), 2.5% ($n = 20$).

Fig. 2 compares the behavioral impact of methanol (0.25% and 2.5%) to that of in the novel object approach test. There was no effect of methanol on time spent in the thigmotaxis zone (Fig. 2A, $P = 0.479$). There was no effect of methanol on time spent in the transition zone (Fig. 2B, $P = 0.490$). There was no effect of methanol on time spent in the center zone (Fig. 2C, $P = 0.678$). Lastly, there was no effect of methanol on distance the fish moved (Fig. 2D, $P = 0.2122$). Data in Fig. 2 are presented as means \pm 1 SEM. Control ($n = 25$), 0.25% ($n = 25$), 2.5% ($n = 20$).

Supplementary data

This is an excel file with the raw data for each variable (time in thigmotaxis zone, time in transition zone, time in center zone, and distance moved) for the 2.5% methanol group and within each test (open field test or novel object approach test). Data for the control and 0.25% methanol groups is available in Hamilton et al., (2021). Raw videos for all groups are available on Dryad.

2. Experimental Design, Materials and Methods

2.1. Fish habitat and husbandry

Adult wild-type (short-fin) zebrafish of mixed sexes (~50:50) were used in this dataset ($n = 70$). Sex was not determined prior to experimentation with individual fish. Zebrafish were obtained from a supplier (Aquatic Imports, Calgary, AB, Canada) and were at least 6 months of age when they were obtained. Fish underwent a 60-day quarantine in the laboratory before being moved to the main habitat. Fish were then held in a three-tier benchtop habitat system (Aquatic Habitats, Aquatic Ecosystems, Inc. Apopka, FL, USA) for at least 30 days prior to experimentation. Fish were housed at a maximum density of 15 fish per 3 L habitat tank. The habitat was maintained at recommended parameters for zebrafish. Temperature was between 28 - 30 °C, pH was 6.5 - 8.0 and dissolved oxygen was at least 5 ppm. Husbandry has been described in previous manuscripts (e.g. [5]). Briefly, on a daily basis ~10% of habitat water was changed and replaced with water containing non-iodized salt, sodium bicarbonate, and acetic acid. Lights were maintained on a 12:12 light/dark cycle with daylight beginning at 8 am. Fish were fed Gemma Micro 300 pellets (Skretting/BioOregon, ME, USA) once per day at approximately noon (\pm 2 h). On experimental days fish were fed after experimentation.

The MacEwan University Animal Research Ethics Board (AREB) approved this research (protocol number 05-12-13) and is in compliance with the Canadian Council for Animal Care (CCAC) guidelines for the care and use of experimental animals.

Open field test

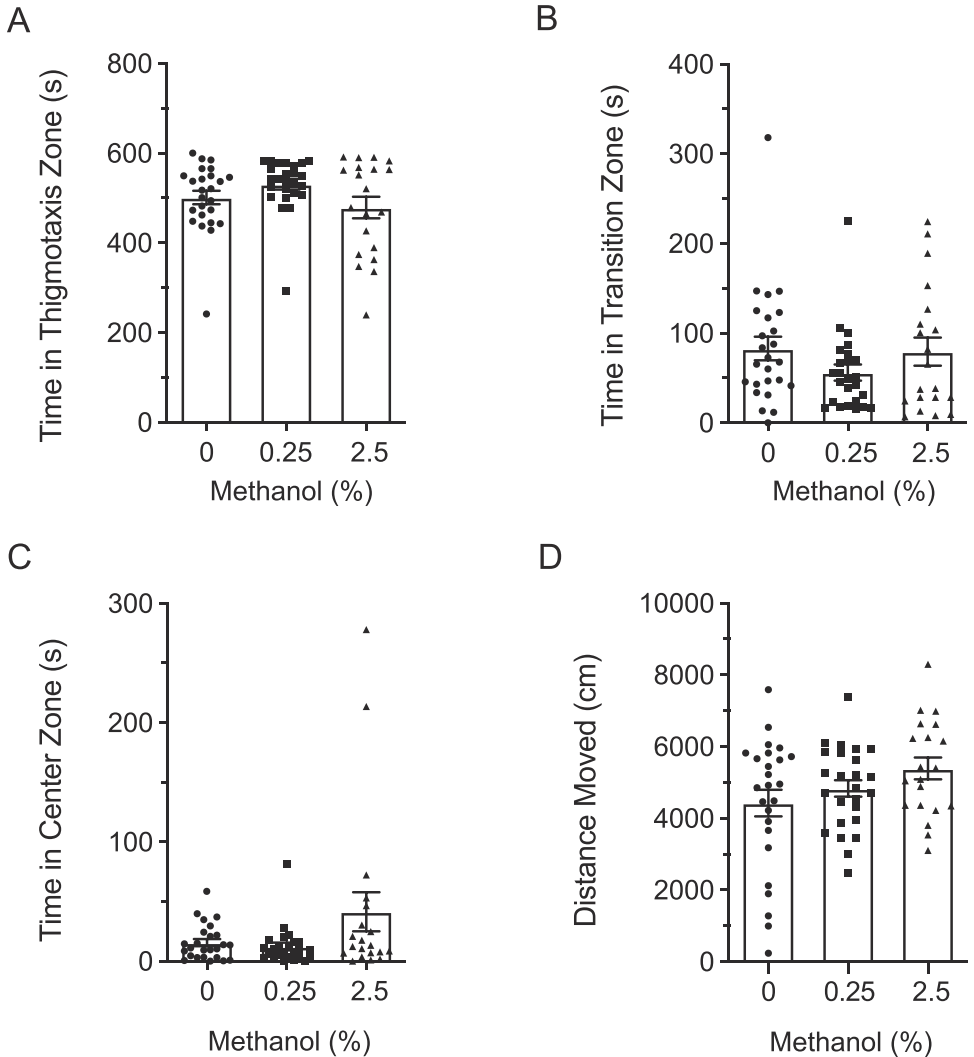


Fig. 1. Methanol effects on behavior in the open field test. Zebrafish were individually exposed to 0, 0.25% or 2.5% methanol before being placed into the open field arena. There was no difference in time spent in the thigmotaxis (A.), transition (B.), or center (C.) zones. There was also no difference in distance moved (D.). Bars are mean \pm SEM and dots, squares, and triangles are individual data points for each fish in the respective groups.

2.2. Methanol exposure

On the day of testing a 3 L tank containing approximately 15 zebrafish was moved to the adjacent testing room. Tanks were placed on seedling heat mats (Hydrofarm Horticultural Products, Petaluma, CA) located on the bench top to maintain the temperature in the habitat tanks and dosing beakers. Fish remained in the habitat tanks for at least 30 min to acclimate to the room and any potential stress due to transport. Next, an individual zebrafish was randomly netted

Novel object approach test

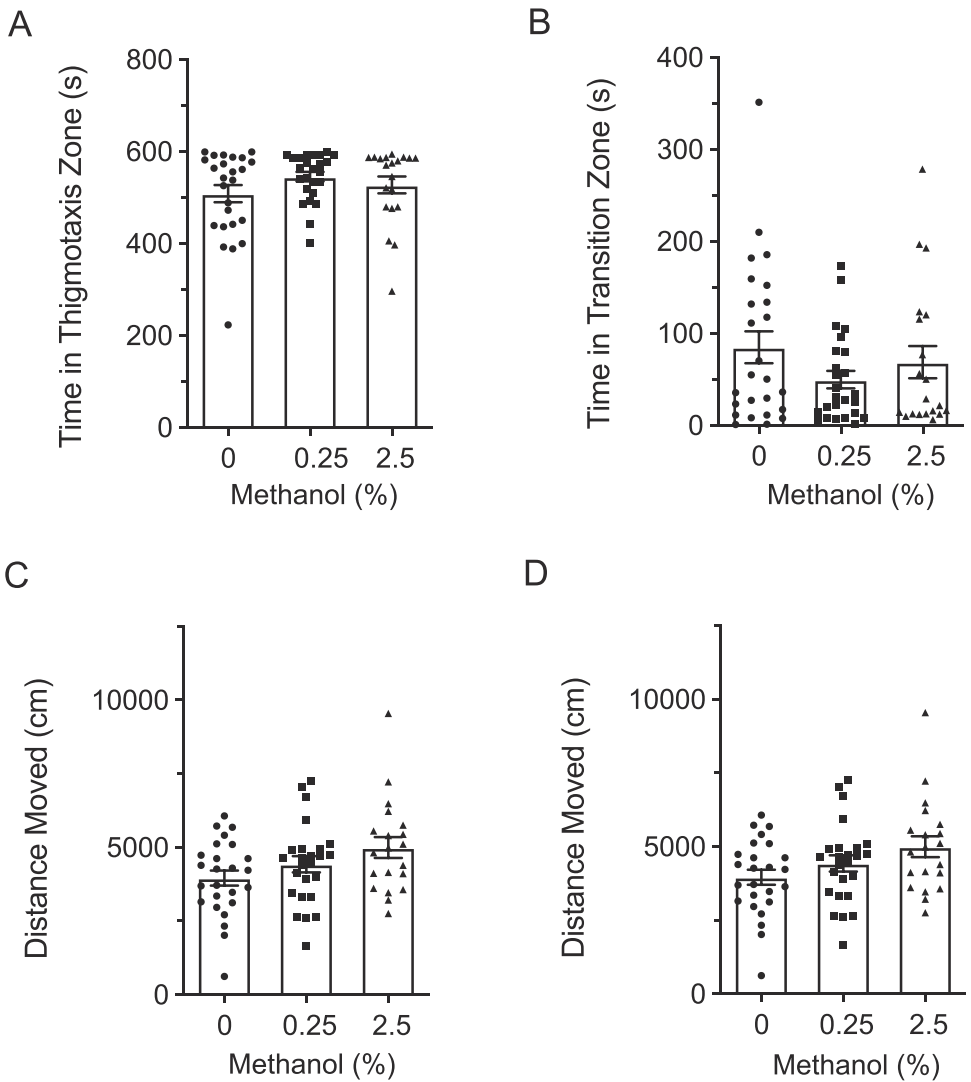


Fig. 2. Methanol effects on behavior in the novel object approach test. Immediately after the open field test ended a novel object was placed in the center of the arena and the novel object approach test began. There was no difference in time spent in the thigmotaxis (A.), transition (B.), or center (C.) zones. There was also no difference in distance moved (D.). Bars are mean \pm SEM and dots, squares, and triangles are individual data points for each fish in the respective groups.

and placed into the dosing 500 ml beaker containing 400 ml of either 0.25% or 2.5% methanol (methyl alcohol, Sigma-Aldrich, Oakville, ON, Canada) in habitat water or habitat water alone (control). These concentrations of methanol were used because they are realistic final concentrations in which methanol can be used as a solvent for B[a]P [6]. White corrugated plastic was used to shield dosing containers and habitats from external stimuli and view of other fish, which

can alter the outcome of behavioral experiments [7]. Individual fish were dosed for 30 min prior to being transferred to the behavioral testing arena.

2.3. Behavioral testing

2.3.1. Open field testing

A very common test to assess locomotion and 'thigmotaxis' – the tendency of the animal to remain close to the wall of an arena – is the open field test. This is a common assay to examine fish behavior in response to toxicants or pharmacological compounds. Increased time spent near the walls is indicative of a potential increase in anxiety-like behavior. In this dataset, fish were placed into the open field arena and were recorded using motion tracking software (EthoVision XT, v. 10, Noldus, VT, USA) for a ten-minute trial. Notably, all fish were released into the arena in an identical manner; fish were allowed to swim out of the net which was placed halfway between the wall and center of the arena, facing the center. This was done to avoid biasing of the fish. Fish behavior was quantified by calculating time fish spent in three virtual zones in the arena. The center zone (innermost zone 0–12 cm in diameter), transition zone (in between the other two zones, 12–23 cm in diameter), and thigmotaxis zones (the outermost zone, 23–34 cm in diameter) were created in EthoVision. Time spent in each of these three zones as well as total distance moved during the trial were quantified for all fish in both behavioral tests. Arena water temperature was checked prior to every open field test and water was replaced following every 4th trial.

2.3.2. Novel object approach testing

To examine 'boldness' or exploration of a novel object, a multicolored LEGO® figurine (4.25 cm tall) was placed into the middle of the arena [7–11] following the open field test. The experimenter, wearing nitrile gloves, slowly affixed the object with Velcro to the arena. Immediately after placing the object into the arena the ten-minute trial began. Like the open field test, the time in zones and distance moved was calculated using the EthoVision XT.

2.4. Statistical analysis

Data was analyzed with Prism (v.9, GraphPad, San Diego, CA, USA). The D'Agostino and Pearson normality test was used to check for normality in all data sets. One-way ANOVAs were used to assess parametric data. Non-parametric data were analyzed with a Kruskal-Wallis test. An alpha level of 0.05 was used for significance. All data are presented as mean \pm SEM.

Ethics Statement

All animal experimentation was approved by the MacEwan University Animal Research Ethics Board (AREB) under protocol number 05–12–13. This is in compliance with the Canadian Council for Animal Care (CCAC) guidelines for the care and use of experimental animals.

CRediT Author Statement

Trevor J. Hamilton: Conceptualization, Methodology, Supervision, Statistical Analysis, Writing; **Joshua Szaszkievicz:** Data collection and statistical analysis; **Jeffrey Krook:** Data collection; **Warren Burggren:** Conceptualization, Methodology, Supervision, Writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.dib.2021.107018](https://doi.org/10.1016/j.dib.2021.107018).

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