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Effects of environmental concentrations of toxins BMAA and its isomers DAB and AEG on zebrafish larvae

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ABSTRACT

The increasing concern over the environmental presence of β-N-Methylamino-L-alanine (BMAA), a toxin primarily produced by cyanobacteria and diatoms, has stimulated numerous studies to evaluate the risk for exposed populations, mainly aquatic organisms and humans. This study focuses on the toxicity of environmental concentrations of BMAA and its isomers, 1-2,4 diaminobutyric acid dihydrochloride (DAB) and N-(2-aminoethyl) glycine (AEG) on zebrafish embryo development (ng.L⁻¹). Presence of BMAA in various environments, including aquatic sources, air, and desert crusts, has raised concerns due to its potential link to neurodegenerative diseases such as the amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC). Despite its known toxicity at high concentrations, there is limited information on the effects of environmental concentrations of BMAA and its isomers. These isomers are often found in association with BMAA and have been detected in seafood intended for human consumption, indicating potential risks from bioaccumulation and biomagnification. Zebrafish embryos have been chosen as a model due to their relevance for embryonic development and toxicity studies. The study employed fish embryo acute toxicity tests and behavioural analyses to specifically assess the sublethal effects of BMAA, DAB, and AEG. The results demonstrated larval mortality rates between 0 % and 3.75 %, while morphological defects were detected across all tested concentrations for each molecule. Behavioural analyses showed alterations in swimming behaviour. Unexpectedly, the changes in morphology and locomotion of the zebrafish larvae were detected more frequently at the lowest concentrations tested, suggesting potential non-monotonic dose responses. Overall, this research underscores the environmental risks associated with BMAA and its isomers, highlighting the importance of continuous monitoring and understanding of their sublethal effects on aquatic organisms and potential implications for human health. Further studies are warranted to elucidate the mechanisms of toxicity, evaluate long-term effects, and assess the risks associated with chronic exposure to these toxins.

1. Introduction

BMAA (β-N-Methylamino-L-alanine) is a toxin mainly produced by cyanobacteria and diatoms (Cox et al., 2005; Violi et al., 2019). It has been identified in several environments and detected in different samples mainly from aquatic sources (Abbes et al., 2022) but also air (Metcalf et al., 2023) and even desert crusts (Metcalf et al., 2015). The interest in studying this toxin is linked to a major human health risk. Several studies showed a possible link to neurodegenerative diseases such as the amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC) (Courtier et al., 2022; Cox et al., 2017). This hypothesis was first postulated after discovering a rare example of extreme

biomagnification at the Guam island where the Chamorro people ingested high doses of the BMAA toxin accumulated in bats that had eaten contaminated cycad seeds. BMAA was measured in bats, as well as in the brains of the Chamorros, and a correlation between cerebral levels of BMAA and neurodegenerative symptoms was demonstrated (Cox et al., 2003). Subsequent studies investigated its toxicity in different animal models (Courtier et al., 2022), while measurements of its presence in the environment were pursued and standardized (Bishop et al., 2020). It should be noted that advances in analytical chemistry, being able to precisely quantify the levels of these molecules in the environment, have been fundamental to a better understanding of the real risk (Pravadali-Cekic et al., 2023). Today, many factors contribute to the

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increased interest in such studies. Although risks of BMAA have been known for a long time, BMAA is considered an emerging risk because its production is increasing due to anthropization of the environment. Human activities, including intensive agricultures that imply the use of chemical fertilizers, together with global warming, are contributing to the extensive production of cyanobacterial blooms worldwide. BMAA and isomers were found at higher concentrations during these efflorescence events (Main et al., 2018; Vo Duy et al., 2019). Abbes et al. (2022) reported in their review concentrations ranging between 10 ng.L^{-1} and $25.3 \text{ } \mu\text{g.L}^{-1}$. These values were obtained after analysis of surface water samples of different origins including lakes and reservoirs from Brazil, Canada, France, Mexico and United Kingdom. Although results might depend on the analysis method used, several works indicate that blooms episodes represent a higher risk, and that the lowest toxins concentrations are in the order of the nanograms per litre (Lopicic et al., 2022). Additionally, BMAA is often found in association with its isomers DAB (L-2,4 diaminobutyric acid dihydrochloride) and AEG (N-(2-aminoethyl) glycine), which have been much less studied and characterized. BMAA has been detected in seafood intended for human consumption in France and other worldwide locations (Banack et al., 2014; Réveillon et al., 2015; Sandhu et al., 2024). This suggests not only a risk associated with direct exposure (water ingestion or air contamination), but also a risk linked to bioaccumulation and biomagnification.

Investigations have proven that BMAA can cause different kinds of toxicity and indicate multiple mechanisms of action that add to the complexity of evaluating the associated risk. As resumed in (Courtier et al., 2022), main pathways of toxicity include oxidative stress, inflammation, lipid synthesis alteration, mitochondrial dysfunctions, glutamatergic system interactions bringing to excitotoxicity, altered protein synthesis and misincorporation and melatonin synthesis inhibition (Li et al., 2024). Together with these pretty well-established mechanisms, an impact on gut microbiome has recently been suggested (Esteves et al., 2023). The authors hypothesize that some Parkinson's Disease cases might be linked to gut microbiome alteration determined by BMAA and warrant the toxin monitoring by public health authorities. To note, there is mounting evidence of the importance of gut microbiome in the development of an organism including the brain (Nunes-Costa et al., 2020), an observation that stands for the importance of a continuous monitoring of possible interfering contaminants. One of the major weaknesses of published toxicology studies is that they analyse the effects of high concentrations that are not relevant to environmental exposure. Therefore, we actually do not know what the consequences of exposure to low concentrations of BMAA might be because only situations of bioaccumulations and biomagnifications have been considered. Furthermore, little is known about the isomers DAB and AEG that are almost always present, and in some cases even represent the major identified form (Abbes et al., 2022; Main et al., 2018; Courtier et al., 2022; Koksharova et al., 2022; Cox, 2021; Metcalf et al., 2023; Amzil et al., 2023). As a consequence, realistic doses of BMAA together with related toxins need to be monitored (Anses, Agence nationale de sécurité sanitaire alimentation, environnement, travail, 2017).

Therefore, in the present study we decided to investigate the toxicity of BMAA and two of its isomers at concentrations going from environmental values up to previously tested doses (from 1 ng.L^{-1} to $500 \text{ } \mu\text{g.L}^{-1}$). Investigating doses in the range of ng.L^{-1} is a necessity because there is a lack in the actual literature. Comparing the results associated to such low concentrations with previously investigated ones will give us the possibility of analysing our study taking into account published data. Several reasons drove the choice of the model tested. Zebrafish are commonly used in toxicology studies, and their embryos are highly amenable to study the dynamic developmental stage that appears to be mostly affected by these compounds. As BMAA has been suggested to be particularly toxic during embryonic development (Powers et al., 2017), we used zebrafish embryos as a model to study the effect of environmental concentrations of BMAA and its isomers on embryos mortality,

development, morphology and locomotion.

2. Materials and methods

2.1. Chemicals

l- β -N-Methylamino-L-alanine hydrochloride (BMAA) (CAS: 16012-55-8), 1-2,4 diaminobutyric acid dihydrochloride (DAB) (CAS: 1883-09-6) and 3,4-Dichloroaniline (DCA) (CAS: 95-76-1) were obtained from Sigma Aldrich (Saint Louis, Missouri, USA), N-(2-aminoethyl) glycine (AEG) (CAS 24123-14-6) was purchased from Santa Cruz Biotechnologies (Dallas, Texas, États-Unis).

A cascade dilution was carried out using a 100 mg.L^{-1} stock solution of each molecule obtained from toxin powder in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl₂, 0.33 mM MgSO₄, methylene blue 0,1 %). The concentrations of the working solutions were 1 ng.L^{-1} , 10 ng.L^{-1} , 100 ng.L^{-1} , $1 \text{ } \mu\text{L.L}^{-1}$ and $500 \text{ } \mu\text{g.L}^{-1}$. DCA (3,4 dichloroaniline) at the concentration of 4 mg.L^{-1} was used as a positive control (OECD, 2013), E3 medium without any addition as a negative control.

2.2. Zebrafish Husbandry and production of embryos

Eggs were obtained from wild-type (AB strain) *Danio rerio*. Zebrafish were kept and handled in compliance with the guidelines of the European Union for handling laboratory animals (https://environment.ec.europa.eu/topics/chemicals/animals-science_en). Conditions of system water were $28 \text{ }^\circ\text{C}$, pH 7.3, conductivity $450 \mu\text{S}$, with a 14 h Light- 10 h dark cycle. The eggs were obtained by natural spawning, and kept in E3 medium in Petri dishes placed in an incubator at $28 \text{ }^\circ\text{C}$. The zebrafish facilities at VBIC are approved by the "Departmental Direction of the Protection of Populations" (DDPP) du Gard (ID 30-189-4). Toxicity studies were not classified as animal experiments according to the 2010/63/EU Directive and were terminated at 4 days post-fertilization (dpf). Embryos were checked and only fertilized eggs that were developing according to established parameters were selected for FET and behavioural investigations.

2.3. Experimental design

The study is divided into two different phases. Firstly, we developed a fish embryo acute toxicity test (FET) associated with a morphological analysis. Secondly, we investigated the locomotion of the larvae using the Daniovision® observation chamber (Noldus Information Technology, Leesburg, VA [www.noldus.com]).

Briefly, embryos were manually dechorionated with fine forceps (Tweezers #5 Dumont) under a stereo microscope at 28 hpf (hours post fertilization) in order to start the exposition to the toxins at the same time and reduce results variability caused by natural dechoriation. FET experiments were inspired by the OECD 236. The pH of each solution was checked before exposure. Only values comprised between 6.5 and 7.5 were accepted and pH was adjusted with HCl or NaOH if necessary (OECD, 2013). Zebrafish larvae were carefully transferred into 48 well-plates after dechoriation, considering 20 larvae for each condition (1 larva/well). Tests were performed under semi-static conditions with refreshment of all media every 24 h and control of pH (Fig. S1). Every experiment was repeated 4 times with different clutches each time, for a total of 80 larvae per condition. Controls are the result of a pool of four groups (20 larvae each) obtained in the 4 biological replicates.

2.3.1. FET fish embryo toxicity

Larvae were maintained at $28 \text{ }^\circ\text{C}$ in the dark except during the observation phases. During media changes, we observed the larvae and noted if any of the following developmental phenotypes had occurred: pericardial edema, yolk sac edema, spinal column disorders (scoliosis), haemorrhage, opacity of the yolk sac or spasms. All observations were

made twice, blinded, by the same observers (Fig. S2).

2.3.2. Behaviour analysis

All testing was performed with the DanioVision behavioural chamber under darkness (infrared light). Larvae were at 72 h post exposition (Fig. S2). Behaviour was registered between 9:00 and 13:00 h. Once placed in the observation chamber, the larvae were acclimatised for 10 min. Then, videos of 5 min were recorded. Three minutes after the beginning a tap was mechanically produced via a solenoid placed at the bottom of the base of the DanioVision chamber. Stimulus intensity was set at the highest level (8 out of 8).

Fish movement was tracked from videos using Ethovision software Version 17.1. Tracks were analysed for a total distance moved (mm) and for the distance moved 1 second after the tap stimulus. To detect larvae, the subtraction method was used with an input filter of 0.5 mm (minimum distance moved) and 5 mm (maximum distance moved) to remove system noises. In the case of persistent artifacts, the concerned larvae were excluded from the analysis.

2.4. Statistics

The percentage of affected larvae for each parameter was determined in the endpoint at 96 hpf (hours post fertilization; 72 h post exposition) for all selected concentrations. Morphological changes were determined using binary choice (present/absent) based on (Frayse et al., 2006). Statistical analyses and graphs were performed using GraphPad Prism (version 10.2.0 for Windows GraphPad Software, Boston, Massachusetts USA, www.graphpad.com). Normality was investigated and Kruskal-Wallis rank-sum tests were applied, followed by Kruskal-Wallis multiple comparison with a Dunn's post hoc tests. To note, normality and homoscedasticity were not satisfied in all samples and only non-parametric tests were applied. Fig. 1 shows the results of different exposures analysing separately morphological effects and dead larvae. For statistical analysis, both were pooled together.

Differences were considered significant if $p < 0.05$ ($ns = p > 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p \leq 0.0001$). Results are shown as the mean \pm SEM (Standard Error of the Mean) unless otherwise indicated.

3. Results

3.1. Morphological effects

In 4 biological replicates, negative control larvae (E3) did not show any malformations or mortality. Conversely, over 95 % of the larvae exposed to DCA (OECD 236 positive controls, DCA = 4 mg.L⁻¹) showed some effects. These larvae are not shown in the graphs presented in

Fig. 1, but in the Supplementary data as they are outside the range of results of the other tested conditions (Fig. S3).

Fig. 1 A shows the results of 72 h exposure to BMAA. At all tested concentrations the larvae showed morphological defects. Interestingly, the incidence of morphological defects increased with decreasing concentrations. Larval death was observed at the concentrations of 1 ng.L⁻¹, 100 ng.L⁻¹ and 500 μ g.L⁻¹. Unexpectedly, the concentration of 1 μ g.L⁻¹ showed the minimum average effect, reproducible over 4 experiments (on average 2,5 % of damaged larvae and no mortality). The concentrations of 100 ng.L⁻¹ and 500 μ g.L⁻¹ induced the maximum average death (3.75 %). When the two observed effects were cumulated (dead larvae and morphologically altered ones), the concentration of 100 ng.L⁻¹ resulted the most impactful. Concerning DAB exposure, we observed that all tested concentrations induced morphological effects (Fig. 1 B) and that only the highest concentrations of 1 and 500 μ g.L⁻¹ induced death of the larvae (2.5 %). The concentration of 1 μ g.L⁻¹ obtained the maximum average of effect over the 4 experiments (5 % of damaged larvae). This same working solution showed the highest effect when considering both dead larvae and morphologically altered ones. Finally, following AEG exposure, all tested concentrations induced morphological defects and caused death of larvae except the 100 ng.L⁻¹ working solution. However, this concentration has the highest average morphological effect. 1 μ g.L⁻¹ and 500 μ g.L⁻¹ solutions showed the maximum average of death (3.75 % of dead larvae). This last concentration showed a statistically significant difference in comparison to E3 controls when morphological effects and dead larvae were pooled together (median = 7,5).

3.2. Behavioural effects

Next, we analysed the effect of toxins on swimming behaviour using the protocol described by Arcanjo and colleagues (Arcanjo et al., 2020). Larvae, placed in E3 medium and in DCA solutions were used as negative and positive controls, respectively. Positive controls are not shown in Fig. 2 because a large majority of DCA-exposed larvae showed disabling morphological damages such as spinal malformations and their swimming behaviour was heavily impacted (Fig. S4).

3.2.1. Total distance moved

After 72 h of exposure to BMAA, only the lowest concentration of 1 ng.L⁻¹ showed a significant difference ($p \leq 0.01$) in the average total distance moved by the larvae compared to unexposed larvae. None of the other concentrations tested reached a statistically significant effect, although they showed the same trend as the 1 ng.L⁻¹, confirming a tendency to reduce the total distance moved. As shown in Fig. 2B, DAB exposure that 10 ng.L⁻¹, 100 ng.L⁻¹ and 1 μ g.L⁻¹ concentrations induced significant differences (respectively $p \leq 0.01$, $p \leq 0.0001$ and p

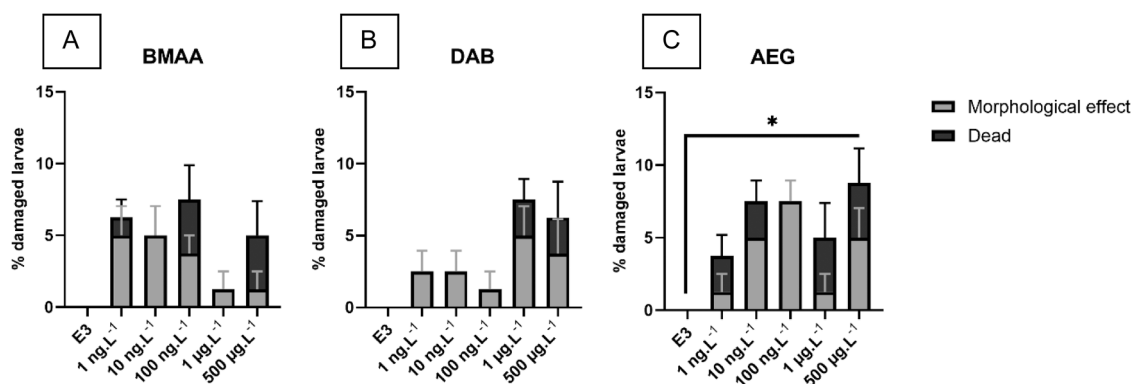


Fig. 1. Average percentage of damaged or dead larvae after 72 h of exposure to different concentrations of BMAA, DAB and AEG for a total of 4 experiments (one experiment = 20 larvae, 4 biological replicates). Mean \pm SEM. Dunn's multiple comparison test results for Kruskal-Wallis analysis of variance on ranks of cumulated data (morphological effect and dead). Statistical icon: * = $P \leq 0.05$. each result following toxin exposure was compared to controls (E3 medium).

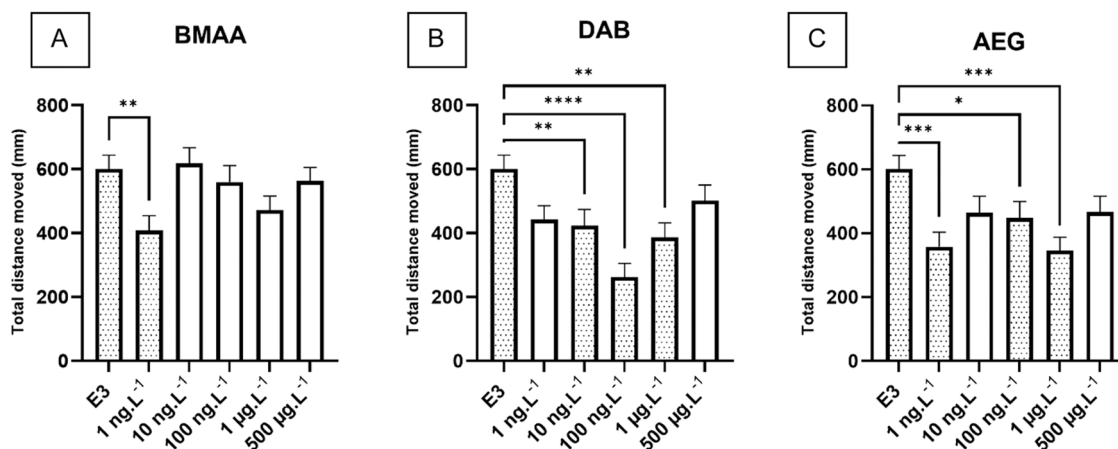


Fig. 2. Total distance moved (mm) by larvae exposed to BMAA, DAB and AEG for 5-min tracks including a tap stimulus (4 biological repeats; larvae with stigma or showing persistent background noise were excluded, $70 < N < 80$). Mean \pm SEM, Dunn's multiple comparison test results for Kruskal-Wallis analysis of variance on ranks. Statistical icon: * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; **** = $P \leq 0.0001$.

≤ 0.01) in the average total distance moved by the larvae compared with unexposed larvae (E3 medium). Three out of the five tested concentrations reached statistical significance. Exposure to 1 ng.L^{-1} , 100 ng.L^{-1} and $1 \mu\text{g.L}^{-1}$ concentrations AEG induced significant differences (Fig. 2C) respectively $p \leq 0.001$, $p \leq 0.05$, $p \leq 0.001$ in the average total distance moved by the larvae compared with unexposed larvae (E3 medium). Three out of five tested concentrations reached statistical significance.

3.2.2. Distance moved 1 s after a tap stimulus

Seventy-two hours after exposure, only a concentration of 1 ng.L^{-1} BMAA induced a significant reduction ($p \leq 0.01$) in the distance moved one second after the tap stimulus, while for DAB 10 ng.L^{-1} and 100 ng.L^{-1} concentrations had significant effects (respectively $p \leq 0.01$, $p \leq 0.0001$) on the distance moved one second after the tap stimulus.

The distance covered by larvae exposed to the toxin is shorter than the distance covered by unexposed ones, similarly to the trend observed for BMAA.

Finally, all tested concentrations of AEG, except $500 \mu\text{g.L}^{-1}$, showed a significant reduction (between $p \leq 0.05$ and $p \leq 0.0001$) in the distance moved one second after the tap stimulus compared to non-treated control larvae.

4. Discussion

The present study focuses on a possible toxicity linked to environmental concentrations of BMAA, DAB and AEG, using zebrafish embryos/larvae. Two different assays have been used, a lethality and morphological analysis, and a behavioural test using the Daniovision® observation chamber. Concerning the morphological analysis, effects were observed at all concentrations for each molecule tested, while no abnormalities were detected in non-treated larvae. Although the percentage of affected larvae was very low, the trends clearly indicate a toxic effect of environmental concentrations of the analysed compounds. Almost every condition (3 molecules and 5 doses) had at least one larva showing a morphological effect, which resulted in death of the larvae in two thirds of the treatments with different concentrations of the molecules studied. We can conclude that in our conditions, the AEG isomer appears to be the most lethal molecule, with four out of five concentrations resulting in the death of some larvae and overall higher morphological effects. To note, a statistically significant difference is observed with $500 \mu\text{g.L}^{-1}$ AEG when morphologically impacted larvae were pooled together with dead ones. The concentrations that were applied in this study are referred to as environmental concentrations because they are those found in several water bodies (Abbes et al.,

2022). Surprisingly, the lowest concentrations tested were not the least effective. In contrast, the concentration of $1 \mu\text{g.L}^{-1}$ of BMAA and 100 ng.L^{-1} DAB appeared to have the smallest effect, as they caused fewer morphological defects and no larval death. In addition, it seems that the correlation between dose and effect is not linear, suggesting the possibility of non-monotonic dose responses (NMDR). Various families of molecules have been described to undergo NMDR. Endocrine disruptors are the most commonly described (Vandenberg et al., 2012). However, other molecules such as copper (O'Doherty et al., 2019), arsenic (Gong et al., 2019) or biopesticides (Souza et al., 2017) are also mentioned in the context of NMDR. This kind of response is starting to be better described and consequently proposed for the interpretation of the effects of molecules showing multiple mechanisms of action (Lagarde et al., 2015; Hill et al., 2018; Lutz et al., 2014; Conolly et Lutz, 2004). As previously indicated, BMAA has been associated to a plethora of different pathways and interactions or superpositions are difficult to predict. Non-linear effects might be the result of an interaction between genetic and environmental factors as hypothesized for the Sod1-G93R zebrafish (Powers et al., 2017). Furthermore, NMDR responses might just be the result of the superposition of different pathways showing different timings. As clearly explained in literature, non-monotonic dose responses studies are difficult to develop and reproduce because slight changes in protocol conditions might bring to completely different results. Discussing limitations of actual protocols, possible improvements and results interpretations will certainly be major issues of future research development in the field. The effects we observed in our study might seem moderate if compared to other toxicity studies, however these were reproducible over 4 biological replicates, performed with different egg clutches at different days. This suggests that the analysed molecules could be prone to NMDR, although further analysis is necessary and our observations need confirmations. To note, we think that the observed differences might be linked to other non-measurable parameters that constitute interindividual differences.

Concerning the behavioural studies conducted on 4 dpf, the larvae exposed to DCA (positive control) exhibited anatomical malformations and travelled a significantly shorter distance than the negative control (Fig. S4). We believe that spinal deformities are the cause of such difference because a large majority of larvae exposed to DCA have some of these alterations (Fig. S2). The effect of toxin exposure was a reduction of the total distance the larvae moved compared to the negative control (E3 medium). Effects were observed for at least one concentration for all toxins (Fig. 2). Statistical analysis revealed significant differences for the lowest concentration of BMAA tested, the three median concentrations of DAB (with high significance for the concentration of 100 ng.L^{-1}), and three concentrations of AEG. None of the highest working solutions

showed any significant difference in the distance covered compared to the control. When considering the distance moved one second after the tap stimulus, the results are almost identical to those observed for the total distance. This includes a reduction in the distance covered with at least one concentration for each molecule (see Fig. 3). The highest concentrations tested are not statistically significant. Under our working conditions, larvae exposed to toxins tended to move less than the control larvae, suggesting they are less reactive to the tap stimulus. Both results from the morphological analysis and the behavioural tests suggest AEG as the most toxic compound, a hypothesis that has already been proposed by another group (Schneider et al., 2020). However, in a different study DAB was shown to be the most toxic, suggesting that experimental conditions might influence the results and that further research into isomers is needed (Martin et al., 2022). A possible non-monotonic dose-response is also highlighted by the various tests we have carried out, although the exact doses and shape of graphs are not identical. Indeed, we must consider that these two analyses are complementary as they are investigating different larvae. In the behavioural tests larvae with stigma or with persistent background noise were excluded.

We know that BMAA has been found to cause hyperexcitability in fish (Courtier et al., 2022). Oxidative stress and excitotoxicity through glutamatergic system interactions has been shown to be an important contributor. There is evidence to support the hypothesis that BMAA acts as an agonist for several glutamate receptors, including NMDA, AMPA, and mGluR5, depending on the form of BMAA (l or d-) (Chiu et al., 2011; Lobner et al., 2007). Knowing that the increase in glutamate in the synapses and the entry of calcium into the post-synaptic terminal can lead to neuronal deterioration (e.g. apoptosis), we believe this might at least partially explain the observed outcomes. Furthermore Méresse and colleagues recently demonstrated that BMAA may target glial cells impacting the central nervous system homeostasis and inducing neuroinflammation (Méresse et al., 2022). Results of behavioural experiments might be different in reported literature, but the exact protocols should be analysed. Recently, Martin and colleagues (Martin et al., 2020) showed an increase in the acoustic startle sensitivity in a dose-dependent manner. However, their tests were conducted at much higher concentrations than those used in this study. The difference between their lowest concentration and ours was one million. Reside et al. (2023) show a moderate increase in overall activity of larvae exposed to $10 \mu\text{g.L}^{-1}$ BMAA under light, and not in dark conditions. They suggest damage to the visual system of zebrafish larvae. Nevertheless, our experiments were carried out entirely in the dark and suggest an effect on larvae comparable to ALS symptoms. Toxicity tests are frequently conducted at concentrations that do not accurately represent actual

environmental exposure. The LC50 (Lethal Concentration 50 %) values reported in the literature often do not correspond to actual exposure levels (e.g. LC50 *Danio rerio* = $10 \mu\text{g.L}^{-1}$ (Purdie et al., 2009) or LC50 *Danio rerio* > 10mg.L^{-1} (Wang, 2015)). The effects observed for LCs can be divisive, as these molecules do not always cause mortality.

These initial results could pave the way for future research on the effects of environmental concentrations of toxins. Non-monotonic dose-response investigations are reported to be particularly difficult in *in vivo* studies (More et al., 2021). However, this is a direction that should be pursued in order to understand the mechanisms of toxicity associated to concentrations in the lower range. *In vitro* as well as *in vivo* and human epidemiological studies should be developed in parallel to confirm the presence of NMDR and consequently evaluate the associated risk (EPA, 2014). These complex studies imply the investigations of multiple conditions that have not been tested so far. For example, an interesting aspect could be to determine the synergistic or antagonistic effects of molecules when present together in mixtures. Martin et al. (2020) found that BMAA and MCLR (microcystin leucine and arginine) interact *in vivo* to enhance neurotoxicity. Another type of study that could be developed concerns our understanding of gene expression variation in zebrafish larvae. As we know that humans share 70 % of orthologous genes with this species, a translational aspect could be considered (Powers et al., 2017).

Finally, evaluating the potential impact of NMDRs in regulatory risk assessment, the EFSA Scientific Committee recommends a “concerted international effort on developing internationally agreed guidance and harmonised frameworks for identifying and addressing NMDRs in the risk assessment process” (More et al., 2021). All studies concerning BMAA and isomers completely apply to such recommendations.

5. Conclusions

The presence of β -N-methylamino-L-alanine (BMAA) and its isomers in the environment has been investigated for decades due to their potential risk to several animal species including humans. Previous studies have exposed models, such as zebrafish, to high concentrations of toxins that are considered unrealistic or only applicable to rare cases of bioaccumulation and biomagnification. It is demonstrated here that doses in the nanogram range have morphological effects and impact the swimming behaviour of larvae. To fully understand the mechanisms of such effects, further investigations are needed. Based on the presented results, the authors underline the possibility of a non-monotonic response and suggest possible research directions.

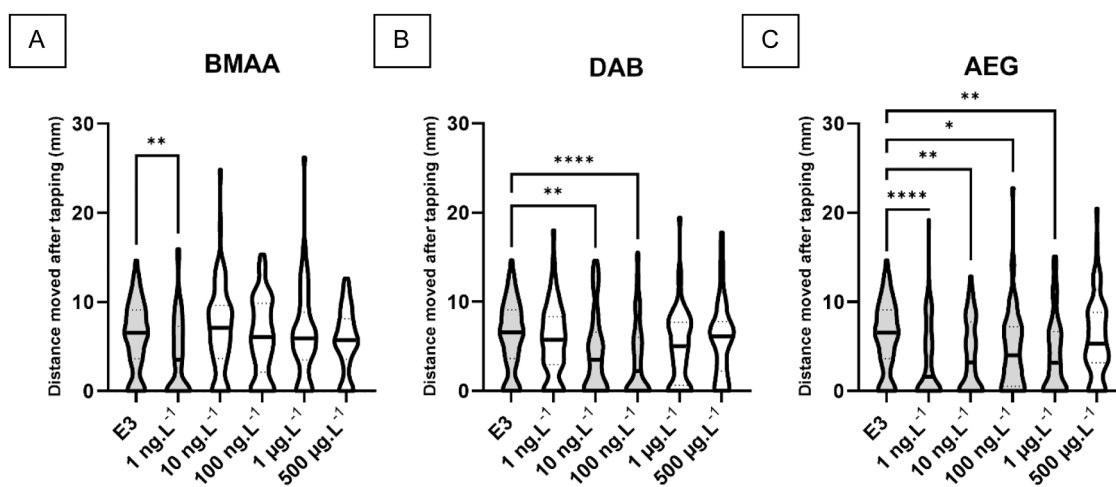


Fig. 3. distance moved (mm) of larvae exposed to BMAA, DAB and AEG 1 s after a tap stimulus (4 biological repeats; larvae with stigma or showing persistent background noise were excluded, $70 < N < 80$). Violin representation, median and quartiles. Dunn's multiple comparison test results for Kruskal-Wallis analysis of variance on ranks. Statistical icon: * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; **** = $P \leq 0.0001$.

CRedit authorship contribution statement

Jeanne Lichtfouse: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Annette C Vergunst:** Writing – review & editing, Resources, Funding acquisition. **Audrey Courtier:** Methodology. **Patrizia Giannoni:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Data availability

All data are available upon request to the authors.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2024.117045](https://doi.org/10.1016/j.ecoenv.2024.117045).

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