BRAIN. Broad Research in Artificial Intelligence and Neuroscience
e-ISSN: 2067-3957 | p-ISSN: 2068-0473
Covered in: Web of Science (ESCI); EBSCO; JERIH PLUS (hkdir.no); IndexCopernicus; Google Scholar; SHERPA/RoMEO; ArticleReach Direct; WorldCat; CrossRef; Peeref; Bridge of Knowledge (mostwiedzy.pl); abcdindex.com; Editage; Ingenta Connect Publication; OALib; scite.ai; Scholar9; Scientific and Technical Information Portal; FID Move; ADVANCED SCIENCES INDEX (European Science Evaluation Centre, neredataltics.org); ivySCI; exaly.com; Journal Selector Tool (letpub.com); Citefactor.org; fatcat!; ZDB catalogue; Catalogue SUDOC (abes.fr); OpenAlex; Wikidata; The ISSN Portal; Socolar; KVK-Volltitel (kit.edu)
2026, Volume 17, Issue 2, pages: 301-315
Submitted: February 24th, 2026 | *Accepted for publication:* May 3rd, 2026

Artificial Intelligence – Assisted Neurobehavioural Assessment of Aquatic Pollutants: Valproic Acid as a Case Study in Zebrafish

Ionut-Alexandru Chelaru
Doctoral School of Geosciences, Faculty of Geography and Geology, "Alexandru Ioan Cuza" University of Iasi, Romania
Department of Biology, Faculty of Sciences, "Vasile Alecsandri" University of Bacau, Romania.
chelaru.alexandru@yahoo.com,
https://orcid.org/0009-0000-0383-1799

Ramona-Alexandra Ciausu*
Doctoral School of Geosciences, Faculty of Geography and Geology, "Alexandru Ioan Cuza" University of Iasi, Romania
ciausuramona05@gmail.com,
https://orcid.org/0009-0001-8262-6538

Mircea Nicusor Nicoara
Doctoral School of Geosciences, Faculty of Geography and Geology, "Alexandru Ioan Cuza" University of Iasi, Romania.
mirmag@uaic.ro,
https://orcid.org/0000-0002-9755-6771

Alin-Stelian Ciobica
Faculty of Biology, "Alexandru Ioan Cuza" University of Iasi, Romania; "Ioan Haulica" Institute, Apollonia University, Iasi, Romania; "Olga Necrasov" Center, Biomedical Research Group, Romanian Academy, Iasi, Romania; Academy of Romanian Scientists, Bucharest Romania; CENEMED Platform for Interdisciplinary Research, University of Medicine and Pharmacy, "Grigore T. Popa", Iași, Romania. alin.ciobica@uaic.ro,
https://orcid.org/0000-0002-1750-6011

Camelia Ureche
Department of Biology, Faculty of Sciences, "Vasile Alecsandri" University of Bacau, Romania. urechec@ub.ro,
https://orcid.org/0009-0004-4133-2121

Abstract: *The increasing presence of pharmaceuticals in aquatic ecosystems has raised significant concerns due to their detrimental effects on both environmental and human health. The present study discusses the rising concern about pharmaceuticals in aquatic environments, focusing on valproic acid (VPA), an antiepileptic drug identified as a neuroactive contaminant. Its persistence in wastewater and limited removal by conventional treatments, along with its known neuroactive properties, prompted the investigation of its neurobehavioural effects in zebrafish (Danio rerio), a model organism for environmental neurotoxicology. Conventional behavioural scoring techniques frequently suffer from subjectivity and inadequate resolution, especially when evaluating the nuanced effects of low-dose exposure or mixture-induced, characteristic in the natural environments. The study highlights the importance of behavioural endpoints as indicators of brain disorders and the role of artificial intelligence (AI) in improving behavioral analysis. By integrating automated video tracking with an AI-assisted exploratory workflow and multivariate analytics, this study illustrates the feasibility of computational approaches for detecting neurobehavioural alterations. After 96 h of exposure, VPA was associated with altered locomotor and spatial behavior in adult zebrafish, evaluated using an optimised low-variance subset (n = 5 per group) within a proof-of-concept framework. These findings highlight the neuroactive potential of VPA and support the use of AI-enhanced zebrafish behavioural models for exploratory environmental neurotoxicology.*

Keywords: *artificial intelligence; neurobehavioural toxicity; zebrafish; emerging pollutants; valproic acid.*

How to cite: Chelaru, I.-A., Ciausu, R.-A., Nicoara, M. N., Ciobica, A.-S., Ureche, C., Andronic, G.-A., & Ureche, D. (2026). Artificial Intelligence–Assisted Neurobehavioural Assessment of Aquatic Pollutants: Valproic Acid as a Case Study in Zebrafish. *BRAIN. Broad Research in Artificial Intelligence and Neuroscience*, *17*(2), 301-315. https://doi.org/10.70594/brain/17.2/18

Gabriel-Andrei Andronic
Department of Biology, Faculty of Sciences, "Vasile Alecsandri" University of Bacau, Romania.
andreigabrielandronic@gmail.com, https://orcid.org/0009-0003-1789-8283

Dorel Ureche
Department of Biology, Faculty of Sciences, "Vasile Alecsandri" University of Bacau, Romania.
dureche@ub.ro, https://orcid.org/0000-0001-9488-9055

1. Introduction

Valproic acid (VPA) is an anticonvulsant and mood stabilising drug, primarily used in the treatment of neurological and psychiatric disorders (Chateauvieux et al., 2010; Felker et al., 2003; Genton et al., 2006; Nanau & Neuman, 2013). The antiepileptic effects are thought to result from suppression of gamma-aminobutyric acid transaminase (GABA-t) activity, along with the modulation of specific neuronal ion channels (Bryson et al., 2023). Through a variety of mechanisms, VPA promotes GABA production, enhancing its release and the postsynaptic GABAergic response (Brahim et al., 2022). In recent years, VPA has been recognised for its ability to inhibit histone deacetylases (HDACs), which are essential enzymes involved in the regulation of chromatin organisation and gene transcription (Göttlicher et al., 2001; Luna-Palencia et al., 2025).

VPA has a relatively acceptable safety profile, in addition to good effectiveness and pharmacoeconomic characteristics. Some patients may occasionally develop serious adverse reactions, such as haemorrhagic pancreatitis, coagulopathies, bone marrow suppression, VPA-induced hepatotoxicity, and encephalopathy; however, the frequency and severity of these unique adverse reactions are still unknown (Ezhilarasan & Mani, 2022; Gayam et al., 2018; Gerstner et al., 2008; Kadam et al., 2025). Furthermore, the use of VPA alone or in combination with other antiepileptic or antipsychotic medicines has been associated with adverse pharmacological responses (Nanau & Neuman, 2013). A sensitive topic is the use of VPA during pregnancy. Similar to other antiepileptic agents, the use of VPA in women of childbearing age is associated with several clinically significant risks and considerations (Genton et al., 2006). Unlike other medications used to control seizures, VPA has not been reported to exhibit pharmacokinetic interactions with other orally administered steroid hormones. Increased risks during pregnancy are expected for both the mother and the developing foetus, as seizure control becomes more challenging. In addition, congenital malformations occurred with the administration of VPA have been reported in scientific articles (Genton et al., 2006; Karlsson Lind et al., 2018).

Reports of an increased risk of neural tube defects (NTDs), particularly spina bifida, in children exposed to VPA during early pregnancy emerged shortly after the drug entered clinical use for the management of epilepsy (Koren et al., 2006). In a subsequent investigation focusing on VPA monotherapy, Jentink et al. (2010) demonstrated that prenatal exposure to VPA was significantly associated with an increased likelihood of 6 out of 14 major congenital malformations. The widespread clinical use of VPA has inevitably contributed to its increasing presence in the environment, as increased pharmaceutical consumption is directly correlated with detectable residues in wastewater systems, as are other pharmaceuticals (Domínguez-García et al., 2024). After administration, pharmaceuticals such as VPA are only partially metabolised in the human body, and a significant fraction is excreted unchanged or as active metabolites through urine and faeces, ultimately accumulating in domestic and hospital wastewater streams (Lunghi et al., 2024).

Recently, the rapid advancement of digital technologies has transformed the landscape of scientific research. Artificial intelligence (AI), machine learning frameworks, and automated data processing tools are now playing an increasingly central role in environmental toxicology, pharmacology, and eco-pharmacovigilance, enabling the analysis of complex datasets generated from chemical monitoring, high-throughput behavioural assays, and molecular profiling (Kleinstreuer & Hartung, 2024; Lin & Chou, 2022; Liu et al., 2024). Advances in digital and computational technologies are increasingly supporting environmental and toxicological research by streamlining evidence collection, improving predictive modelling, and strengthening risk assessment workflows through sophisticated analytical tools (Paut Kusturica et al., 2022). In aquatic models, AI-assisted behavioural tracking methods now allow the detection of subtle neurobehavioural effects in zebrafish with much greater accuracy than manual observation alone. Together, these developments complement traditional experimental approaches and provide a more robust framework for evaluating the ecological implications of pharmaceutical contaminants such as VPA. Integrating such methodologies enhances our ability to characterise environmental risks and supports ongoing efforts to improve monitoring and mitigation strategies. In aquatic models,

AI-assisted analytical tools enable more sensitive detection of toxic effects, thereby strengthening risk assessment frameworks and improving the evaluation of pharmaceutical pollution processes. The following illustration provides an integrated overview of how environmental pharmaceutical contaminants, such as VPA released through wastewater effluents, can be assessed for their neurobehavioural toxicity using a zebrafish model combined with AI-assisted analyses (Figure 1).

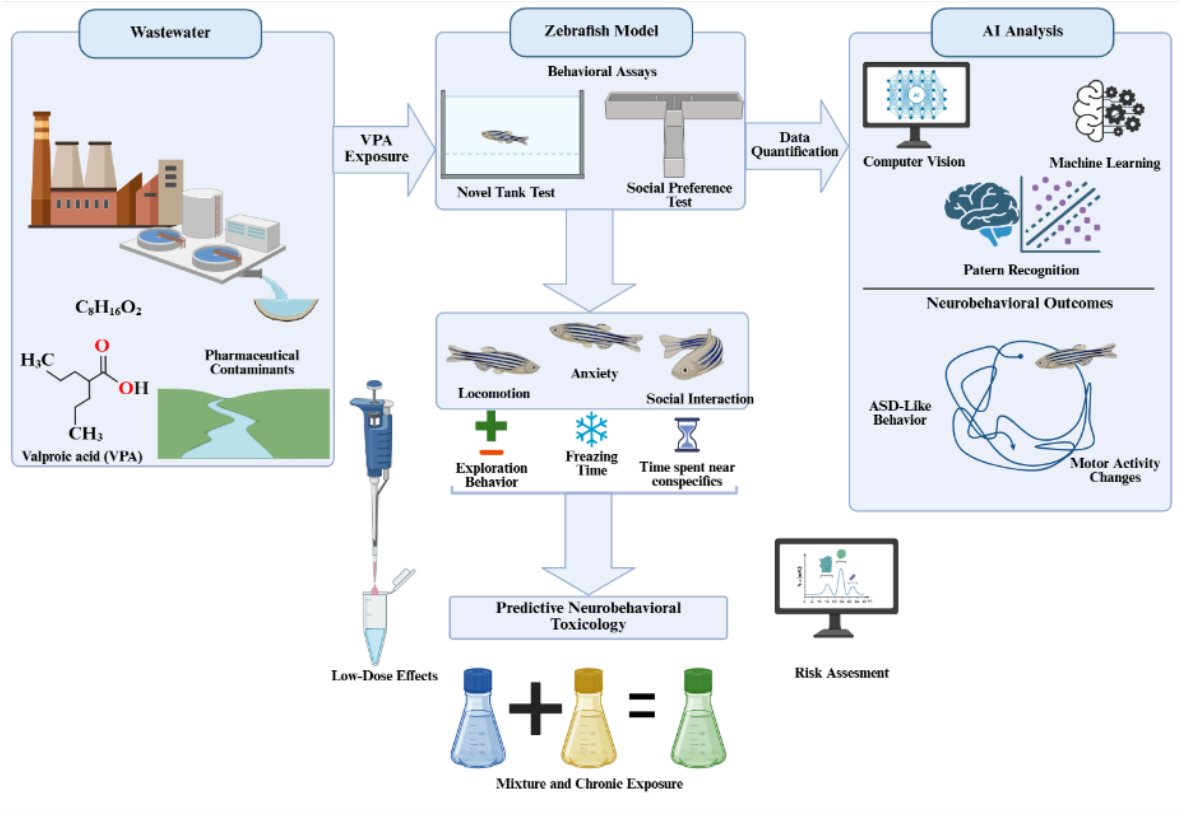

Figure 1. Integration of zebrafish model for neurobehavioural toxicology assessment

Note. Reproduced from *Integration of zebrafish model for neurobehavioral toxicology assessment*, by I.-A. Chelaru, 2026, BioRender (https://biorender.com/j0x7c5h). Created with BioRender.com.

2. Valproic Acid as a Neuroactive Pollutant

2.1. Occurrence and Persistence in Wastewater

VPA is a commonly prescribed antiepileptic drug found in aquatic systems globally. Like other pharmaceuticals, it mainly enters surface waters through human excretions, discharges from wastewater treatment plants (WWTPs), agricultural runoff, and improper disposal of pharmaceuticals (Cardoso-Vera et al., 2021; Heberer, 2002). A status report by UNESCO and HELCOM (2017) confirmed that VPA is one of the most widely sold pharmaceuticals (25,274 kg $year^{-1}$), ranking 8^{th} in the Baltic Sea basin. Due to its extensive clinical use and low biodegradability, VPA is often detected in wastewater influents and effluents.

Since conventional wastewater treatment methods are not specifically designed to remove pharmaceutically active compounds (Chaber-Jarlachowicz et al., 2025; Coimbra et al., 2021), VPA and other pharmaceuticals often persist after treatment processes and are discharged into receiving surface waters, including rivers, lakes, and other freshwater bodies. Monitoring studies have confirmed that pharmaceuticals, including widely used antiepileptic drugs, can be detected at concentrations ranging from ng L^{-1} to µg L^{-1} in treated effluents and downstream environments, highlighting the limitations of current treatment technologies. Measured concentrations of VPA in wastewater and surface waters show notable spatial variations. At the Back River wastewater

treatment plant in Baltimore, influent samples contained approximately 130 ng L^{-1} of VPA (Yu et al., 2012), while in Lake Mälaren in Sweden, levels of up to 2.6 µg L^{-1} were reported (Rehrl, 2019).

Chronic release of VPA into surface waters raises concerns due to its neuroactive effects on aquatic fauna. Despite environmental concentrations below therapeutic doses, continued exposure alongside other neuroactive pharmaceuticals could have significant impacts on non-target aquatic organisms (Jarema et al., 2022). Consequently, VPA is recognized as an emerging contaminant of concern, linking pharmaceutical pollution to environmental neurotoxicology.

2.2. Neuroactive Properties of VPA

VPA is a broad-spectrum antiepileptic drug that modulates inhibitory neurotransmission and epigenetic regulation. It enhances GABA signalling by inhibiting GABA transaminase, increasing the synaptic availability of GABA and reducing neuronal excitability (Vinken, 2013). In addition, VPA serves as an HDAC inhibitor, which alters chromatin structure and gene expression important for neurodevelopment and synaptic plasticity (Göttlicher et al., 2001). These mechanisms, although beneficial in clinical contexts, pose risks when VPA contaminates the environment, affecting non-target organisms. In aquatic species, especially during early development, VPA can disrupt GABAergic signalling and epigenetic processes, leading to problems in neuronal differentiation, circuit formation and behaviour (Ton et al., 2006; Zarate-Lopez et al., 2024).

Regarding the exposure of complex aquatic organisms such as zebrafish to VPA, changes in locomotor activity, increased anxiety-like behaviour, and impaired social interactions have been observed, demonstrating significant neurobehavioural disruptions (MacPhail et al., 2009; Ton et al., 2006). Developmental exposure to VPA in zebrafish serves as a model for neurodevelopmental disorders, especially autism spectrum disorder (ASD)-like phenotypes, due to conserved molecular and behavioural pathways across vertebrates (Jarema et al., 2022; Ricarte et al., 2024). This highlights the neuroactive potency of VPA at both low or high concentrations and its relevance for assessing neurobehavioural toxicity in environmental or neurotoxicological studies.

Overall, the combined influence of VPA on neurotransmitter pathways and epigenetic mechanisms makes it a particularly noteworthy neuroactive contaminant of emerging concern. Its well-characterised mechanisms of action, combined with measurable behavioural outcomes in zebrafish, make VPA an ideal candidate for AI-assisted neurobehavioural analyses aimed at detecting subtle, chronic, or mixture-induced effects of pharmaceutical contaminants in aquatic environments.

3. Zebrafish as a Model for Environmental Neurotoxicity

The presence of VPA in aquatic environments raises concerns about chronic exposure in aquatic ecosystems, where VPA residues have already been shown to affect various biological indicators, including aquatic plants such as *Lemna minor* (Machado et al., 2023). Although primary producers such as *Lemna minor* provide valuable early signals of environmental contamination, assessing the ecological implications of VPA requires examining its effects on more complex aquatic organisms such as zebrafish.

Zebrafish (*Danio rerio)* represents a valuable organism model, with conserved neurological, cardiovascular, and genetic homology with humans and developmental pathways that enable the detection of subtle toxicological outcomes (Angom & Nakka, 2024; Burgess & Burton, 2023; Nishimura et al., 2015).

Behaviour serves as a comprehensive manifestation that indicates the functional output of the neurological system. Zebrafish exhibit a diverse range of measurable behaviours, encompassing locomotion, anxiety-like reactions, social interactions, learning, and sensorimotor integration. These behaviours are governed by conserved neurotransmitter systems and neural circuits, enabling neurobehavioural alterations to act as sensitive indicators of central nervous system disruptions due to environmental contaminants, including pharmaceuticals, pesticides, and industrial chemicals (Kalueff et al. 2014; Tierney, 2011). Zebrafish are also particularly well-suited for investigating

environmentally relevant exposure scenarios, such as chronic low-dose exposure and complex chemical combinations commonly found in wastewater effluents. behavioural assays in zebrafish have demonstrated sensitivity to pharmacological combinations at concentrations below those causing overt toxicity, underlining their importance for environmental risk assessment (Jarema et al., 2022). Furthermore, zebrafish models are well-suited for high-throughput experimental designs due to their small size, low maintenance, and compatibility with automation, enabling the simultaneous testing of multiple individuals and extensive collection of behavioural data (Kalueff, Stewart, & Gerlai, 2014). This scalability requires advanced analytical tools for large, complex data, where artificial intelligence-based approaches provide significant advantages.

4. Neurobehavioural Effects of Valproic Acid in Zebrafish

Zebrafish (*Danio rerio*) serves as an effective model for studying the neurobehavioural impacts of VPA, driven by conserved neurodevelopmental pathways and genetic accessibility. Exposure to VPA during developmental stages correlates with significant changes in locomotor activity, anxiety-like behaviour, and impaired social interaction, indicating a disruption of the central nervous system (Ricarte et al., 2024; Ton, Lin, & Willett, 2006). In this context, results using laboratory models further support this, showing that early exposure to VPA interferes with neuronal development in ways that later become visible at both behavioural and morphological levels.

Exposure of zebrafish to VPA during embryonic or larval stages results in significant neurobehavioural changes, including altered locomotion, thigmotaxis, and exploratory behaviour, suggesting impacts on motor function and anxiety (MacPhail et al., 2009; Ton et al., 2006). These behavioural effects correlate with molecular and neuroanatomical changes that influence neurogenesis and synaptic development, reflecting the epigenetic and neurotransmitter-modulating effects of VPA (Kim et al., 2025).

Researchers have found that zebrafish exposed to VPA during key neurodevelopmental stages display behaviours similar to autism spectrum disorder (ASD), such as decreased social interaction and altered locomotion (Jarema et al., 2022; Ricarte et al., 2024). Studies show that zebrafish embryos exposed to VPA during early life later develop autism-like behaviour, including altered sensory processing, hyperactivity, disruption of neurotransmitter balance, and lasting social deficits. For this reason, zebrafish are widely used as an experimental model for autism research, as VPA-induced alterations reliably reproduce the core neurobehavioural features relevant to ASD, including social, sensory, and neurochemical disturbances (Ricarte et al., 2024). For example, Ricarte et al. demonstrated that exposing zebrafish embryos to VPA during the 4-48 h post-fertilisation (hpf) period induces persistent neurodevelopmental alterations. Larvae displayed increased locomotor activity, reduced responsiveness to visual and vibrational stimuli, and dysregulation of neurotransmitters, including increased levels of glutamate and reduced levels of acetylcholine and norepinephrine. These early disruptions extended into adulthood, where previously exposed fish exhibited atypical social behaviour, characterised by less cohesive shoaling and diminished interest in conspecifics, along with reduced levels of cerebral dopamine and GABA (Ricarte et al., 2024). Furthermore, the study by Zimmermann et al. (2015) showed a deficit in social interaction at 70 and 120 days post-fertilisation (dpf) after exposure to VPA (48 μM). Similarly, Baronio et al. (2018) reported that 25 μM VPA administered between 10 and 24 hpf subsequently impaired the social preference of adult zebrafish at 6 months post fertilisation (mpf). Together, these findings illustrate how routine therapeutic use of VPA ultimately contributes to its environmental fate, emphasising the need for improved removal strategies and broader ecopharmacovigilance efforts. This consistency across studies underscores the potential of VPA-exposed zebrafish as a model for studying neurodevelopmental toxicity.

Exposure to VPA beyond the early developmental stages results in long-lasting neurobehavioural effects in juvenile and adult zebrafish, including hypoactivity, hyperactivity, altered stress responses, and memory deficits (Kalueff et al., 2014; Ton et al., 2006). These observations indicate that early disruptions in neurodevelopment may produce effects that persist

long after exposure has ended. Behavioural assays, such as the novel tank test, the social preference test, and the light-dark preference test, are used to assess neurotoxicity (Kalueff et al., 2014). These assays produce sensitive readouts as well as large, complex datasets that are difficult to interpret with traditional manual scoring methods.

Concerns about the neurobehavioural effects of VPA in zebrafish arise from its detection at trace levels in surface waters and wastewater. Even low-dose exposure can result in subtle and significant behavioural changes, indicating the sensitivity of zebrafish as a marker for neuroactive pollutants. For example, recent research from our group has demonstrated that a 96-hour exposure to VPA in adult zebrafish resulted in a mild but statistically significant reduction in social behaviour, both when administered alone and in combination with other pharmaceutical compounds (Chelaru et al., 2025). This emphasises the need for analytical methods that can detect these minor phenotypic changes (Jarema et al., 2022). VPA induces diverse neurobehavioural effects in zebrafish, highlighting the value of the model in studying pharmaceuticals. However, the complexity of behavioural responses reveals the limitation of traditional analysis methods, leading to the need for AI-based tools for objective and scalable behavioural phenotyping.

5. Artificial Intelligence in Neurobehavioural Analysis, Zebrafish Models, and Wastewater Neurotoxicology

Conventional behavioural scoring in zebrafish predominantly depends on manual observation or rudimentary tracking systems that assess movement, distance travelled, immobility, and angular variations. While useful for fundamental toxicity assessments, these methods are constrained by observer bias, inadequate temporal resolution, and the incapacity to identify nuanced or intricate behavioural abnormalities (Fontana et al., 2025b). This constraint is particularly pertinent when assessing the impacts of VPA, which elicits subtle alterations in locomotor activity, anxiety-like behavior, and social interaction that may elude traditional methods (Rihel et al., 2010).

AI provides revolutionary answers for these issues. Computer vision algorithms can derive high-resolution movement trajectories and posture data from video recordings, collecting detailed behavioural events over extensive populations and prolonged observation periods (Yang et al., 2021). Machine learning algorithms, such as support vector machines, random forests, and k-nearest neighbours, facilitate the classification of treatment groups based on behavioural markers, yielding objective and reproducible phenotyping (Fontana et al., 2025a). Importantly, deep learning techniques such as convolutional neural networks (CNNs) and recurrent neural networks (RNNs) enable the detection of latent patterns across spatiotemporal dimensions, supporting advanced pattern recognition and analysis in neurobehavioural data (Nascu, 2025; Prasad et al., 2025).

AI also enables high-throughput behavioural screening, allowing for the assessment of low-dose and chronic exposure effects, as well as mixture interactions encountered in environmental situations (Fontana et al., 2025a). This method is especially relevant for VPA, which persists in wastewater at low concentrations but can cause cumulative neurobehavioural alterations in aquatic organisms. Zebrafish behavioural datasets can be used to measure neurotoxic consequences by combining AI-driven tracking, machine learning-based categorisation, and deep learning pattern recognition (Rihel et al., 2010). AI enables researchers to progress from simple locomotor measures to high-dimensional neurobehavioural phenotyping, providing sensitive, reproducible, and translationally relevant data for environmental neurotoxicology studies (Fontana et al., 2025a; Yang et al., 2021).

The combination of AI, zebrafish models, and wastewater neurotoxicology results in a predictive and translational framework for evaluating the neurobehavioural impact of new pollutants like VPA. Zebrafish serve as an exemplary model for identifying neurodevelopmental and behavioural alterations induced by pollutants due to their conserved neuroanatomy, rapid development, and varied behavioural repertoire (Rihel et al., 2010).

Behavioural assays in zebrafish, such as locomotion tracking, anxiety-like behaviour tests, and social interaction assays, can be used to detect neurotoxicity (Dutra Costa et al., 2020). AI

improves the sensitivity and impartiality of these tests by automating tracking, feature extraction, and pattern identification. Machine learning classifiers, for example, can distinguish VPA-induced behavioural profiles from controls, but deep learning models can detect small changes that indicate neurodevelopmental disturbance (Bozhko et al., 2022).

Researchers can develop predictive neurobehavioural models by integrating the following components: wastewater sampling identifies the presence and concentration of neuroactive contaminants, zebrafish assays demonstrate functional neurobehavioural outcomes, and AI analyses offer high-dimensional, reproducible, and scalable quantification of effects (Fontana et al., 2025b). VPA is used as a reference neuroactive substance to evaluate this framework, proving the ability of AI-enhanced zebrafish models to identify modest neurobehavioural changes under relevant environmental exposure scenarios (Fontana et al., 2025b; Rihel et al., 2010).

This comprehensive approach offers a robust foundation for tackling numerous persistent challenges in environmental neurotoxicology. Researchers can identify modest neurobehavioural changes resulting from low-dose, chronic, or mixed chemical exposures, which are frequently missed by traditional scoring methods (Kalueff et al., 2014; Tierney, 2011), by integrating zebrafish behavioural models with AI-driven analytical tools. The implementation of automated, data-driven behavioural analysis fosters uniformity and reproducibility of tests among laboratories, mitigating observer bias and improving cross-study comparability (Gomez-Marin et al., 2014; Pereira et al., 2020). Furthermore, AI-assisted extraction of high-dimensional behavioural patterns makes it easier to translate aquatic neurobehavioural data into predictive insights for human neurotoxicity, enhancing the translational potential of zebrafish-based models (MacRae & Peterson, 2015; Wiltschko et al., 2015). The scalability of AI-driven processes facilitates the construction of high-throughput screening pipelines, which allow for efficient prioritisation and risk assessment of emerging pollutants in complex aquatic matrices (Guo et al., 2025; Küster & Adler, 2014).

Finally, the combination of AI, zebrafish models, and wastewater neurotoxicology provides a solid foundation for predictive environmental risk assessment, directing both research and regulatory decision-making for neuroactive pollutants (Rihel et al., 2010).

6. The Exploratory Role of Artificial Intelligence in Neurobehavioural Analysis: Valproic Acid as a Case Study in Zebrafish

Behavioural effects of VPA (3 μg L^{-1}) were assessed in adult zebrafish following 96 h of exposure using the novel tank test. Locomotor and exploratory behaviours were quantified by automated video tracking with EthoVision XT 17. Data preprocessing and analysis were performed within a Python-based computational environment. AI-assisted tools, including Microsoft Copilot, were used to support code development, optimisation, and workflow automation. Importantly, all analytical procedures relied on predefined statistical and variance-based criteria; AI tools did not perform statistical inference, sample selection decisions, or result interpretation.

To evaluate and reduce technical variability, a multivariate variance-based exploratory procedure was applied to the full dataset. For each experimental group, variance and dispersion across multiple behavioural endpoints were calculated, and all possible combinations of five individuals were assessed. The subset exhibiting the lowest within-group multivariate variance was retained as a representative, low-variance dataset for downstream comparative analysis (n = 5 per group). Selection was independent of treatment outcome, effect direction, or endpoint magnitude, and no raw data were excluded.

This dataset included five established zebrafish behavioural endpoints that represent locomotor activity, exploratory behaviour, and anxiety-related responses (Fig. 2): (a) Total Distance Travelled (cm), (b) Velocity (cm/s), (c) Thigmotaxis Cumulative Duration (s), (d) Centre Cumulative Duration (s), and (e) Immobility Cumulative Duration (s). Each parameter was represented by a combined box-and-jitter plot, which displayed individual data points as well as group distributions.

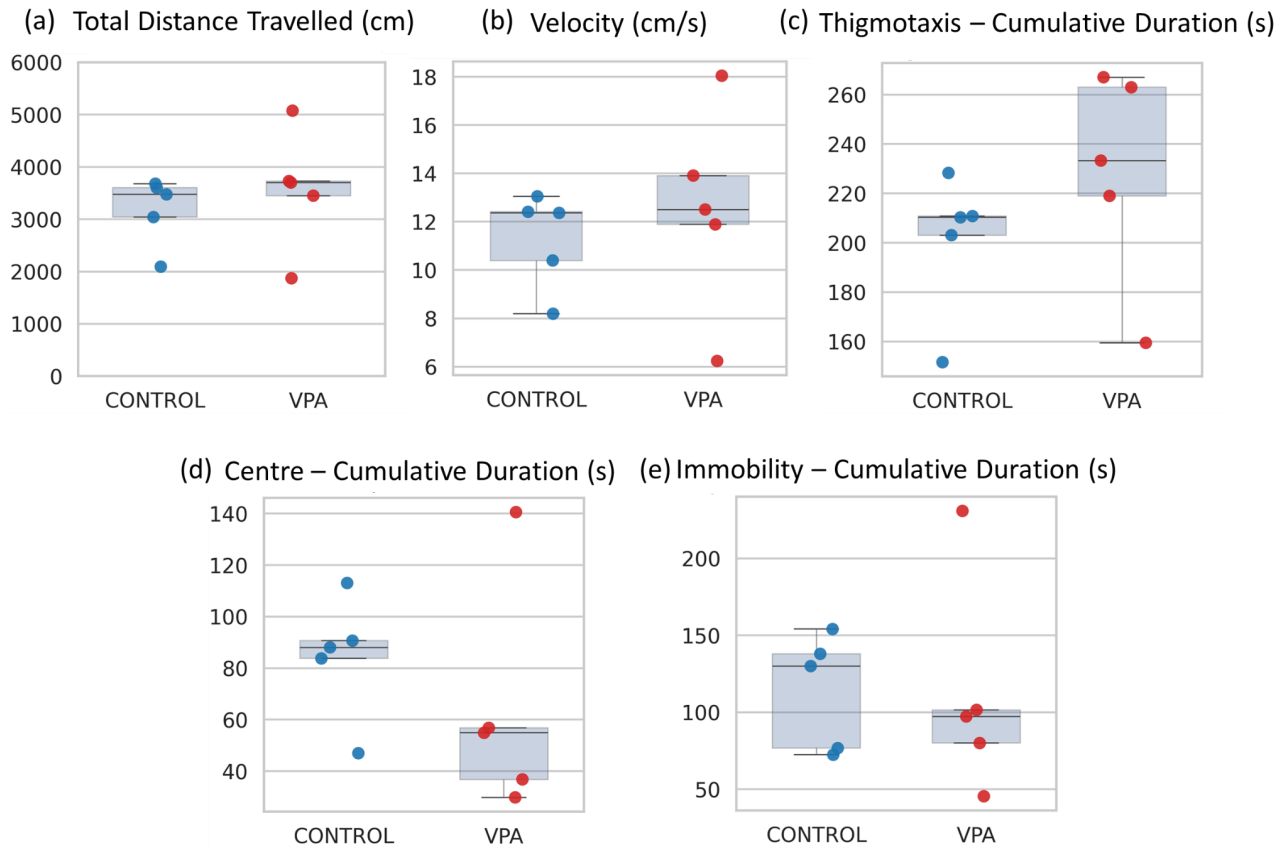

Figure 2. Exploratory, locomotor, and anxiety-related behavioural endpoints quantified in the optimised dataset (n = 5 CONTROL; n = 5 VPA), with data presented as mean ± SD, after 96 h exposure. The main parameters measured include: (a) Total Distance Travelled (cm), (b) Velocity (cm/s), (c) Thigmotaxis Cumulative Duration (s), (d) Centre Cumulative Duration (s), and (e) Immobility Cumulative Duration (s).

Across all behavioural endpoints, the VPA-exposed group had a consistent and coherent neurobehavioural pattern. VPA administration resulted in a hyper locomotor response, with adults travelling substantially longer distances and swimming faster than controls. Furthermore, VPA-exposed adults exhibited lower immobility, indicating heightened motor activation and decreased behavioural inhibition. Similar results have been reported in studies conducted on zebrafish adults (Dhanabal & Durairaj, 2024; Chelaru et al., 2025). Concomitantly, VPA administration resulted in significant changes in spatial preference, including increased thigmotaxis and decreased centre zone exploration, indicating an anxiogenic behavioural shift. As Borba et al. (2025) affirmed, thigmotaxis represents the fish preference for the edge of the aquarium, an endpoint that reflects the relationship between anxiogenic- and anxiolytic-like behaviour.

VPA-exposed individuals displayed reduced multivariate dispersion relative to controls, facilitating clearer visualisation of group-level behavioural trends. Collectively, these findings demonstrate that VPA elicits robust, multidomain neurobehavioural alterations in zebrafish. By integrating AI-assisted exploratory preprocessing with optimised sample selection, we substantially reduced behavioural noise and may support the detection of pollutant-induced neurobehavioural alterations, enabling precise detection of VPA-induced hyperactivity, increased peripheral occupation, reduced centre exploration, and diminished immobility. These results highlight the power of artificial intelligence–enhanced behavioural analysis as a sensitive and scalable framework for characterising pollutant-induced neurobehavioural disruption in aquatic toxicology research.

7. Limitations and Future Directions

Despite the intriguing combination of AI, zebrafish models, and wastewater neurotoxicology for detecting the neurobehavioural consequences of new pollutants and their mixtures, numerous limitations constrain present research and point to distinct future possibilities.

The quality and uniformity of behavioural datasets present a fundamental challenge. AI and machine learning models rely heavily on large, representative datasets for robust training and generalisation, but many zebrafish behavioural studies use small sample sizes, inconsistent annotation standards, and variable protocols. Manual categorisation of behaviours is time-consuming, subjective, and subject to inter-annotator disagreement, reducing repeatability across laboratories (Creton, 2009; Segalin et al., 2021). The research made by Fazzari et al. (2025) conducted a comprehensive evaluation of deep learning-based methods for animal behaviour analysis, emphasising issues with data quality, annotation, and model generalisability. Environmental and husbandry factors, including water chemistry, tank geometry, and lighting, can greatly influence behavioural outcomes in zebrafish research. These conditions are usually underreported, hindering study comparability and replication (Hillman et al., 2024).

Although AI approaches provide enhanced pattern recognition capabilities, they also present issues in model interpretability and generalisability. Many deep learning architectures, such as convolutional and recurrent neural networks, which are commonly used in animal behaviour analysis, operate as "black boxes," providing little insight into how decisions are made or which features drive classification outcomes (Hassija et al., 2024). The opacity of these models can hinder biological interpretation of AI-detected phenotypes, complicating efforts to link behavioural signals to underlying neural mechanisms (Elshawi et al., 2019). Overfitting is a concern for models trained on small or biased datasets, as performance on training data may not translate to new, real-world conditions. This limitation is emphasised across AI applications in biomedical domains (Rizzo et al., 2025).

Furthermore, algorithmic bias, where training data inadvertently embed systematic biases (e.g., resulting from unique laboratory circumstances or experimental designs), can distort model outputs and impair generalisability (Mavrogiorgos et al., 2024).

The findings of our experimental dataset reveal VPA as a key neuroactive pollutant of concern, emphasising the importance of AI-enhanced zebrafish behavioural models for environmental risk assessment and prediction neurotoxicology. However, one significant limitation of this work is the use of an optimised low-variance subset (n = 5 per group), reflecting its proof-of-concept and methodological focus. While variance-informed preprocessing facilitated clearer visualisation of behavioural trends by reducing technical noise, the small sample size limits statistical power and precludes population-level generalisation. The AI-assisted pipeline was implemented as a deterministic, exploratory tool and was not intended for predictive modelling or autonomous decision-making; therefore, behavioural heterogeneity remains an important consideration. Future studies should validate this framework using larger cohorts and independent datasets to assess robustness, scalability, and applicability across different neuroactive pollutants and behavioural paradigms.

The biological intricacy of zebrafish behaviour and its interpretation impose inherent restrictions. While zebrafish are valuable translational models, their neuroanatomical simplicity and the sensitivity of behavioural endpoints to external stressors necessitate caution in interpreting certain phenotypes such as "anxiety-like" or cognitive behaviours (Chauhan et al., 2026). Furthermore, innovative behavioural paradigms, such as learning or operant conditioning procedures may have low replicability when transferred between laboratories, highlighting the importance of rigorous validation and refinement (Agrillo et al., 2024). AI-driven analysis faces practical constraints such as technical skills and processing infrastructure. Many laboratories lack the computational skills and resources required to set up and maintain complex machine learning processes. Open-access tools, such as stance-tracking software, may require technical expertise that is not broadly available (Teicher et al., 2025).

To optimise the impact of AI-enhanced zebrafish neurobehavioural research, several strategic initiatives need to be followed.

The first step is to create uniform, large-scale behavioural datasets. Shared annotation frameworks and multi-laboratory benchmarking will allow for more robust AI training, cross-study comparisons, and improved repeatability across laboratories (Creton, 2009; Fazzari et al., 2025; Pereira et al., 2020).

Second, the discipline should use hybrid analytical frameworks to combine AI-extracted behavioural traits with molecular, neurophysiological, or imaging data. Such multimodal techniques will improve mechanistic understanding and translational relevance by connecting reported behavioural phenotypes to the underlying neural circuits (Chauhan et al., 2026; Tierney, 2011).

Third, experimental designs must account for environmentally realistic exposures. Fourth, ongoing investment in open-source AI tools and collaboration platforms is essential. Lowering technical obstacles will provide access to advanced behavioural analytical methodologies, allowing for wider use and community-wide data sharing (Guo et al., 2025; Wiltschko et al., 2015). Finally, rigorous validation and openness should be prioritized. Efforts to improve model explainability, such as the use of interpretable machine learning techniques, will ensure that AI outputs are biologically meaningful and actionable, hence facilitating regulatory decision-making (Hassija et al., 2024; Mavrogiorgos et al., 2024).

By pursuing these directions, the field can make progress toward AI-enhanced zebrafish neurobehavioural evaluation that is robust, reproducible, and ecologically relevant, laying the groundwork for better environmental monitoring, neurotoxicology research, and translational neuroscience applications.

8. Conclusions

VPA is a neuroactive pharmaceutical that remains in aquatic environments due to extensive use and inadequate removal by wastewater treatment. Its environmental persistence, stable chemical properties, and known neuropharmacological effects raise significant concern regarding aquatic neurotoxicity, making it a key reference contaminant for environmental studies. Evidence from zebrafish models indicates that low concentrations of VPA exposure can lead to significant changes in locomotion, anxiety-like behaviour, social preference, and neurodevelopment. These neurobehavioural outcomes reflect brain disruptions sensitive to pharmaceutical exposure but are complex and subtle, complicating traditional behavioural assessment methods.

AI serves as a transformative tool in environmental neurotoxicology by enabling objective and detailed analysis of behavior. This high-resolution approach aids in identifying subtle effects from low-dose exposures and mixtures that might be missed. When combined with zebrafish models, AI enhances the connection between behavioural phenotyping and insights into human neurotoxicity. Together, the integration of AI, zebrafish as a neurobehavioural model, and wastewater-derived neuroactive contaminants like VPA represents a significant advancement in neurotoxicity assessment.

Future studies on standardisation, transparency, and multimodal integration are crucial for developing effective tools for environmental monitoring, regulatory decision-making, and translational neuroscience.

References

Agrillo, C., Rovegno, E., Dadda, M., Bertolucci, C., & Bisazza, A. (2024). Assessment of replicability and efforts to refine an operant conditioning procedure for larval zebrafish. *Animals, 14*(24), 3684. https://doi.org/10.3390/ani14243684

Angom, R. S., & Nakka, N. M. R. (2024). Zebrafish as a model for cardiovascular and metabolic disease: The future of precision medicine. *Biomedicines, 12*(3), 693. https://doi.org/10.3390/biomedicines12030693

Baronio, D., Puttonen, H. A. J., Sundvik, M., Semenova, S., Lehtonen, E., & Panula, P. (2018). Embryonic exposure to valproic acid affects the histaminergic system and the social behaviour of adult zebrafish (*Danio rerio*). *British Journal of Pharmacology, 175*(5), 797–809. https://doi.org/10.1111/bph.14124

Borba, J. V., Resmim, C. M., Gonçalves, F. L., Silva, R. M., Pretzel, C. W., Moraes, H. S., Sauter, M. D., & Rosemberg, D. B. (2025). Anxiety modulators elicit different behavioral outcomes in adult zebrafish: Emphasis on homebase-related parameters and spatio-temporal exploration. *Pharmacology, Biochemistry, and Behavior, 246*, 173914. https://doi.org/10.1016/j.pbb.2024.173914

Bozhko, D. V., Myrov, V. O., Kolchanova, S. M., Polovian, A. I., Galumov, G. K., Demin, K. A., Zabegalov, K. N., Strekalova, T., de Abreu, M. S., Petersen, E. V., & Kalueff, A. V. (2022). Artificial intelligence-driven phenotyping of zebrafish psychoactive drug responses. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 112*, 110405. https://doi.org/10.1016/j.pnpbp.2021.110405

Brahim, S., Henia, M., Mohamed, A. H., Aoun, M. H., Zarrouk, L., Orsolini, L., Bellagamba, S., Longo, G., Tempia Valenta, S., Salvi, V., & Volpe, U. (2022). Growing use of valproic acid in substance use disorders. *European Psychiatry, 65*(S1), S243–S244. https://doi.org/10.1192/j.eurpsy.2022.628

Bryson, A., Reid, C., & Petrou, S. (2023). Fundamental neurochemistry review: GABAA receptor neurotransmission and epilepsy: Principles, disease mechanisms and pharmacotherapy. *Journal of Neurochemistry, 165*(1), 6–28. https://doi.org/10.1111/jnc.15769

Burgess, H. A., & Burton, E. A. (2023). A critical review of zebrafish neurological disease models—1. The premise: Neuroanatomical, cellular and genetic homology and experimental tractability. *Oxford Open Neuroscience, 2*, kvac018. https://doi.org/10.1093/oons/kvac018

Cardoso-Vera, J. D., Elizalde-Velázquez, G. A., Islas-Flores, H., Mejía-García, A., Ortega-Olvera, J. M., & Gómez-Oliván, L. M. (2021). A review of antiepileptic drugs: Part 1 occurrence, fate in aquatic environments and removal during different treatment technologies. *Science of the Total Environment, 768*, 145487. https://doi.org/10.1016/j.scitotenv.2021.145487

Chaber-Jarlachowicz, P., Gworek, B., & Kalinowski, R. (2025). Removal efficiency of pharmaceuticals during the wastewater treatment process: Emission and environmental risk assessment. *PLOS ONE, 20*(9), e0331211. https://doi.org/10.1371/journal.pone.0331211

Chateauvieux, S., Morceau, F., Dicato, M., & Diederich, M. (2010). Molecular and therapeutic potential and toxicity of valproic acid. *Journal of Biomedicine and Biotechnology, 2010*, 479364. https://doi.org/10.1155/2010/479364

Chauhan, A., Raviraj, T., Sobti, R. C., Dutta, A., Shukla, M. K., & Korinek, M. (2026). Zebrafish models for neurological disorders: A platform for natural product-based drug discovery. *Frontiers in Natural Products, 4*, 1679580. https://doi.org/10.3389/fntpr.2025.1679580

Chelaru, I.-A. (2026). *Integration of zebrafish model for neurobehavioral toxicology assessment*. BioRender. https://biorender.com/j0x7c5h

Chelaru, I.-A., Strungaru-Jijie, R., Nicoara, M., Mirila, D., Ciobica, A., & Ureche, D. (2025). Single and combined effects of meropenem, valproic acid, and ketoprofen on adult zebrafish behavior, oxidative stress, and acetylcholinesterase activity. *Pharmaceuticals, 18*(8), 1096. https://doi.org/10.3390/ph18081096

Coimbra, R. N., Escapa, C., & Otero, M. (2021). Removal of pharmaceuticals from water: Conventional and alternative treatments. *Water, 13*(4), 487. https://doi.org/10.3390/w13040487

Creton, R. (2009). Automated analysis of behavior in zebrafish larvae. *Behavioural Brain Research, 203*(1), 127–136. https://doi.org/10.1016/j.bbr.2009.04.030

Dhanabal, M., & Durairaj, B. (2024). Valproic acid induced autism-like behaviour in zebrafish—chrysin as a miracle cure. *Indian Journal of Pharmaceutical Sciences, 86*(1). https://doi.org/10.36468/pharmaceutical-sciences.1286

Domínguez-García, P., Aljabasini, O., Barata, C., & Gómez-Canela, C. (2024). Environmental risk assessment of pharmaceuticals in wastewaters and reclaimed water from Catalan main river basins. *Science of the Total Environment, 949*, 175020. https://doi.org/10.1016/j.scitotenv.2024.175020

Dutra Costa, B. P., Aquino Moura, L., Gomes Pinto, S. A., Lima-Maximino, M., & Maximino, C. (2020). Zebrafish models in neural and behavioral toxicology across the life stages. *Fishes, 5*(3), 23. https://doi.org/10.3390/fishes5030023

Elshawi, R., Al-Mallah, M. H., & Sakr, S. (2019). On the interpretability of machine learning-based model for predicting hypertension. *BMC Medical Informatics and Decision Making, 19*(1), 146. https://doi.org/10.1186/s12911-019-0874-0

Ezhilarasan, D., & Mani, U. (2022). Valproic acid induced liver injury: An insight into molecular toxicological mechanism. *Environmental Toxicology and Pharmacology, 95*, 103967. https://doi.org/10.1016/j.etap.2022.103967

Fazzari, E., Romano, D., Falchi, F., & Stefanini, C. (2025). Animal behavior analysis methods using deep learning: A survey. *Expert Systems with Applications, 289*, 128330. https://doi.org/10.1016/j.eswa.2025.128330

Felker, B. L., Sloan, K. L., Dominitz, J. A., & Barnes, R. F. (2003). The safety of valproic acid use for patients with hepatitis C infection. *American Journal of Psychiatry, 160*(1), 174–178. https://doi.org/10.1176/appi.ajp.160.1.174

Fontana, B. D., Blanco, L., Uchoa, A. E., Müller, M. L., Gonçalves, F. L., Resmim, C. M., Borba, J. V., Canzian, J., & Rosemberg, D. B. (2025a). Development and applications of a machine learning model for an in-depth analysis of pentylenetetrazol-induced seizure-like behaviors in adult zebrafish. *Neuroscience, 568*, 377–387. https://doi.org/10.1016/j.neuroscience.2025.01.048

Fontana, B. D., Canzian, J., & Rosemberg, D. B. (2025b). Swimming into the future: Machine learning in zebrafish behavioral research. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 139*, 111398. https://doi.org/10.1016/j.pnpbp.2025.111398

Gayam, V., Mandal, A. K., Khalid, M., Shrestha, B., Garlapati, P., & Khalid, M. (2018). Valproic acid induced acute liver injury resulting in hepatic encephalopathy—A case report and literature review. *Journal of Community Hospital Internal Medicine Perspectives, 8*(5), 311–314. https://doi.org/10.1080/20009666.2018.1514933

Genton, P., Semah, F., & Trinka, E. (2006). Valproic acid in epilepsy: Pregnancy-related issues. *Drug Safety, 29*(1), 1–21. https://doi.org/10.2165/00002018-200629010-00001

Gerstner, T., Bell, N., & König, S. (2008). Oral valproic acid for epilepsy—Long-term experience in therapy and side effects. *Expert Opinion on Pharmacotherapy, 9*(2), 285–292. https://doi.org/10.1517/14656566.9.2.285

Gomez-Marin, A., Paton, J. J., Kampff, A. R., Costa, R. M., & Mainen, Z. F. (2014). Big behavioral data: Psychology, ethology and the foundations of neuroscience. *Nature Neuroscience, 17*(11), 1455–1462. https://doi.org/10.1038/nn.3812

Göttlicher, M., Minucci, S., Zhu, P., Krämer, O. H., Schimpf, A., Giavara, S., Sleeman, J. P., Lo Coco, F., Nervi, C., Pelicci, P. G., & Heinzel, T. (2001). Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO Journal, 20*(24), 6969–6978. https://doi.org/10.1093/emboj/20.24.6969

Guo, Z., Huang, L., Yan, J., Zhang, H., Jia, X., Li, M., & Li, H. (2025). Artificial intelligence technology in environmental research and health: Development and prospects. *Environment International, 203*, 109788. https://doi.org/10.1016/j.envint.2025.109788

Hassija, V., Chamola, V., Mahapatra, A., Singal, A., Goel, D., Huang, K., Scardapane, S., & Hussain, A. (2024). Interpreting black-box models: A review on explainable artificial intelligence. *Cognitive Computation, 16*(1), 45–74. https://doi.org/10.1007/s12559-023-10179-8

Heberer, T. (2002). Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: A review of recent research data. *Toxicology Letters, 131*(1–2), 5–17. https://doi.org/10.1016/s0378-4274(02)00041-3

Hillman, C., Fontana, B. D., Amstislavskaya, T. G., Gorbunova, M. A., Altenhofen, S., Barthelson, K., Bastos, L. M., Borba, J. V., Bonan, C. D., Brennan, C. H., Farias-Cea, A., Cooper, A., Corcoran, J., Dondossola, E. R., Martinez-Duran, L. M., Gallas-Lopes, M., Galstyan, D. S., Garcia, E. O., Gerken, E., Hindges, R., … Parker, M. O. (2024). Housing and husbandry factors affecting zebrafish (*Danio rerio*) novel tank test responses: A global multi-laboratory study. *Research Square*. https://doi.org/10.21203/rs.3.rs-4849877/v1

Jarema, K. A., Hunter, D. L., Hill, B. N., Olin, J. K., Britton, K. N., Waalkes, M. R., & Padilla, S. (2022). Developmental neurotoxicity and behavioral screening in larval zebrafish with a comparison to other published results. *Toxics, 10*(5), 256. https://doi.org/10.3390/toxics10050256

Jentink, J., Loane, M. A., Dolk, H., Barisic, I., Garne, E., Morris, J. K., & de Jong-van den Berg, L. T. W. (2010). Valproic acid monotherapy in pregnancy and major congenital malformations. *New England Journal of Medicine, 362*(23), 2185–2193. https://doi.org/10.1056/NEJMoa0907328

Kadam, R., Palkar, M., & Pingili, R. B. (2025). Mechanisms involved in the valproic acid-induced hepatotoxicity: A comprehensive review. *Toxicology Mechanisms and Methods, 35*(6), 565–580. https://doi.org/10.1080/15376516.2025.2459176

Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in Pharmacological Sciences, 35*(2), 63–75. https://doi.org/10.1016/j.tips.2013.12.002

Karlsson Lind, L., Komen, J., Wettermark, B., von Euler, M., & Tomson, T. (2018). Valproic acid utilization among girls and women in Stockholm: Impact of regulatory restrictions. *Epilepsia Open, 3*(3), 357–363. https://doi.org/10.1002/epi4.12228

Kim, J., Park, S. H., & Sun, W. (2025). The differential developmental neurotoxicity of valproic acid on anterior and posterior neural induction of human pluripotent stem cells. *International Journal of Stem Cells, 18*(1), 49–58. https://doi.org/10.15283/ijsc24066

Kleinstreuer, N., & Hartung, T. (2024). Artificial intelligence (AI)—it's the end of the tox as we know it (and I feel fine). *Archives of Toxicology, 98*(3), 735–754. https://doi.org/10.1007/s00204-023-03666-2

Koren, G., Nava-Ocampo, A. A., Moretti, M. E., Sussman, R., & Nulman, I. (2006). Major malformations with valproic acid. *Canadian Family Physician, 52*(4), 441-447. https://pmc.ncbi.nlm.nih.gov/articles/PMC1481679/

Küster, A., & Adler, N. (2014). Pharmaceuticals in the environment: Scientific evidence of risks and its regulation. *Philosophical Transactions of the Royal Society B: Biological Sciences, 369*(1656), 20130587. https://doi.org/10.1098/rstb.2013.0587

Lin, Z., & Chou, W. C. (2022). Machine learning and artificial intelligence in toxicological sciences. *Toxicological Sciences, 189*(1), 7–19. https://doi.org/10.1093/toxsci/kfac075

Liu, J. Y., Peeples, J., & Sayes, C. M. (2024). Evaluation of machine learning based QSAR models for the classification of lung surfactant inhibitors. *Environment & Health, 2*(12), 912–917. https://doi.org/10.1021/envhealth.4c00118

Luna-Palencia, G. R., Correa-Basurto, J., & Vásquez-Moctezuma, I. (2025). Valproic acid as a histone deacetylase inhibitor induces ABCB1 overexpression and de novo ABCB5 expression in HeLa cells. *Current Issues in Molecular Biology, 47*(9), 749. https://doi.org/10.3390/cimb47090749

Lunghi, C., Valetto, M. R., Caracciolo, A. B., Bramke, I., Caroli, S., Bottoni, P., Castiglioni, S., Crisafulli, S., Cuzzolin, L., Deambrosis, P., Giunchi, V., Grisotto, J., Marcomini, A., Moretti, U., Murgia, V., Pandit, J., Polesello, S., Poluzzi, E., Romizi, R., … Paolone, G. (2024). Call

to action: Pharmaceutical residues in the environment: Threats to ecosystems and human health. *Drug Safety, 48*(4), 315–320. https://doi.org/10.1007/s40264-024-01497-3

Machado, B., Prudêncio, C., Ferraz, R., & Barros, P. (2023). Ecotoxicity of valproic acid in *Lemna minor*. *Proceedings of Research and Practice in Allied and Environmental Health, 1*(1), 23. https://doi.org/10.26537/prpaeh.v1i1.5179

MacPhail, R. C., Brooks, J., Hunter, D. L., Padnos, B., Irons, T. D., & Padilla, S. (2009). Locomotion in larval zebrafish: Influence of time of day, lighting and ethanol. *Neurotoxicology, 30*(1), 52–58. https://doi.org/10.1016/j.neuro.2008.09.011

MacRae, C. A., & Peterson, R. T. (2015). Zebrafish as tools for drug discovery. *Nature Reviews Drug Discovery, 14*(10), 721–731. https://doi.org/10.1038/nrd4627

Mavrogiorgos, K., Kiourtis, A., Mavrogiorgou, A., Menychtas, A., & Kyriazis, D. (2024). Bias in machine learning: A literature review. *Applied Sciences, 14*(19), 8860. https://doi.org/10.3390/app14198860

Nanau, R. M., & Neuman, M. G. (2013). Adverse drug reactions induced by valproic acid. *Clinical Biochemistry, 46*(15), 1323–1338. https://doi.org/10.1016/j.clinbiochem.2013.06.012

Nascu, A. (2025). Region of interest detection using AI techniques and morphological operations. *BRAIN. Broad Research in Artificial Intelligence and Neuroscience, 16*(2), 418–434. https://doi.org/10.70594/brain/16.2/30

Nishimura, Y., Murakami, S., Ashikawa, Y., Sasagawa, S., Umemoto, N., Shimada, Y., & Tanaka, T. (2015). Zebrafish as a systems toxicology model for developmental neurotoxicity testing. *Congenital Anomalies, 55*(1), 1–16. https://doi.org/10.1111/cga.12079

Paut Kusturica, M., Jevtic, M., & Ristovski, J. T. (2022). Minimizing the environmental impact of unused pharmaceuticals: Review focused on prevention. *Frontiers in Environmental Science, 10*, 1077974. https://doi.org/10.3389/fenvs.2022.1077974

Pereira, T. D., Shaevitz, J. W., & Murthy, M. (2020). Quantifying behavior to understand the brain. *Nature Neuroscience, 23*(12), 1537–1549. https://doi.org/10.1038/s41593-020-00734-z

Prasad, P., A. J., Zakharov, A., Romanchuk, N., & Hemanth, D. (2025). CNLNet: Enhancing MRI brain image denoising using a convolutional neural network with integrated non-local means layer. *BRAIN. Broad Research in Artificial Intelligence and Neuroscience, 16*(2), 335–352. https://doi.org/10.70594/brain/16.2/24

Rehrl, A.-L. (2019). *Occurrence and fate of organic micropollutants (OMPs) in Lake Mälaren* (Second cycle, A2E). Swedish University of Agricultural Sciences, Department of Aquatic Sciences and Assessment. https://stud.epsilon.slu.se/14185/

Ricarte, M., Tagkalidou, N., Bellot, M., Bedrossiantz, J., Prats, E., Gomez-Canela, C., Garcia-Reyero, N., & Raldúa, D. (2024). Short- and long-term neurobehavioral effects of developmental exposure to valproic acid in zebrafish. *International Journal of Molecular Sciences, 25*(14), 7688. https://doi.org/10.3390/ijms25147688

Rihel, J., Prober, D. A., Arvanites, A., Lam, K., Zimmerman, S., Jang, S., Haggarty, S. J., Kokel, D., Rubin, L. L., Peterson, R. T., & Schier, A. F. (2010). Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science, 327*(5963), 348–351. https://doi.org/10.1126/science.1183090

Rizzo, M., Veneri, A., Marcuzzo, M., Zangari, A., Albarelli, A., Lucchese, C., Nobile, M. S., & Conati, C. (2025). Machine learning models explanations as interpretations of evidence: A theoretical framework of explainability and its implications on high-stakes biomedical decision-making. *BMC Medical Research Methodology, 25*(Suppl 1), 282. https://doi.org/10.1186/s12874-025-02703-1

Segalin, C., Williams, J., Karigo, T., Hui, M., Zelikowsky, M., Sun, J. J., Perona, P., Anderson, D. J., & Kennedy, A. (2021). The mouse action recognition system (MARS) software pipeline for automated analysis of social behaviors in mice. *eLife, 10*, e63720. https://doi.org/10.7554/eLife.63720

Teicher, G., Riffe, R. M., Barnaby, W., Martin, G., Clayton, B. E., Trapani, J. G., & Downes, G. B. (2025). Marigold: A machine learning-based web app for zebrafish pose tracking. *BMC Bioinformatics, 26*(1), 30. https://doi.org/10.1186/s12859-025-06042-2

Tierney, K. B. (2011). Behavioural assessments of neurotoxic effects and neurodegeneration in zebrafish. *Biochimica et Biophysica Acta, 1812*(3), 381–389. https://doi.org/10.1016/j.bbadis.2010.10.011

Ton, C., Lin, Y., & Willett, C. (2006). Zebrafish as a model for developmental neurotoxicity testing. *Birth Defects Research Part A: Clinical and Molecular Teratology, 76*(7), 553–567. https://doi.org/10.1002/bdra.20281

UNESCO, & HELCOM. (2017). *Pharmaceuticals in the aquatic environment of the Baltic Sea region: A status report* (Emerging Pollutants in Water Series, No. 1). UNESCO Publishing. https://unesdoc.unesco.org/ark:/48223/pf0000247889

Vinken, M. (2013). The adverse outcome pathway concept: A pragmatic tool in toxicology. *Toxicology, 312*, 158–165. https://doi.org/10.1016/j.tox.2013.08.011

Wiltschko, A. B., Johnson, M. J., Iurilli, G., Peterson, R. E., Katon, J. M., Pashkovski, S. L., Abraira, V. E., Adams, R. P., & Datta, S. R. (2015). Mapping sub-second structure in mouse behavior. *Neuron, 88*(6), 1121–1135. https://doi.org/10.1016/j.neuron.2015.11.031

Yang, P., Takahashi, H., Murase, M., & Itoh, M. (2021). Zebrafish behavior feature recognition using three-dimensional tracking and machine learning. *Scientific Reports, 11*(1), 13492. https://doi.org/10.1038/s41598-021-92854-0

Yu, J. T., Bisceglia, K. J., Bouwer, E. J., Roberts, A. L., & Coelhan, M. (2012). Determination of pharmaceuticals and antiseptics in water by solid-phase extraction and gas chromatography/mass spectrometry: Analysis via pentafluorobenzylation and stable isotope dilution. *Analytical and Bioanalytical Chemistry, 403*(2), 583–591. https://doi.org/10.1007/s00216-012-5846-5

Zarate-Lopez, D., Torres-Chávez, A. L., Gálvez-Contreras, A. Y., & Gonzalez-Perez, O. (2024). Three decades of valproate: A current model for studying autism spectrum disorder. *Current Neuropharmacology, 22*(2), 260–289. https://doi.org/10.2174/1570159x22666231003121513

Zimmermann, F. F., Gaspary, K. V., Leite, C. E., De Paula Cognato, G., & Bonan, C. D. (2015). Embryological exposure to valproic acid induces social interaction deficits in zebrafish (*Danio rerio*): A developmental behavior analysis. *Neurotoxicology and Teratology, 52*(Pt A), 36–41. https://doi.org/10.1016/j.ntt.2015.10.002