ACTA VET. BRNO 2025, 94: 231–242; https://doi.org/10.2754/avb202594030231

Therapeutic use meets environmental concern: Gabapentin's toxicological profile in aquatic ecosystems – a review

Renáta Hesová, Zdeňka Svobodová, Pavla Lakdawala

University of Veterinary Sciences Brno, Faculty of Veterinary Hygiene and Ecology,
Department of Animal Protection and Welfare and Veterinary Public Health, Brno, Czech Republic

Received May 28, 2025
Accepted September 5, 2025

Abstract

Gabapentin (GBP) is a widely prescribed antiepileptic and analgesic drug that has increasingly been detected in wastewater and surface water due to its incomplete metabolism and the fact that it is not efficiently removed by conventional wastewater treatment plants (WWTPs). Its persistence in the aquatic environment has raised concerns due to its possible impact on aquatic organisms. Typical environmental concentrations of GBP range from tens to hundreds of ng/l, with maximums of several µg/l near WWTP effluents. While GBP is not acutely toxic at these amounts, sublethal effects have been observed in aquatic species, particularly in the zebrafish (*Danio rerio*), common carp (*Cyprinus carpio*) and the daphnids (*Daphnia magna*). Reported impacts include disrupted cardiovascular development, oxidative stress, apoptosis, altered gene expression related to immunity and neurodevelopment, and metabolic disturbances. In zebrafish embryos, concentrations of GBP as low as 0.1 µg/l have been associated with enzymatic changes and vascular impairment. Efforts to mitigate the environmental impact of GBP include exploring advanced processing methods for removing GBP from wastewater, such as ozonisation. However, transformation products such as GBP-L may exhibit greater persistence and toxicity than the parent compound. Therefore, further research is urgently needed to understand the fate of GBP in the environment and to develop effective strategies for its removal and risk management. This comprehensive review highlights the dual significance of gabapentin, emphasising its therapeutic potential and the need to address its environmental implications for sustainable healthcare and ecological management.

Antiepileptics, Danio rerio, fish, aquatic environment

Gabapentin (GBP), also known as neurotin or 1-(aminomethyl)cycloheaxaneacetic (Macdonald and Kelly 1995; Mao and Chen 2000; Gilron and Flatters 2006; NCBI 2024), was first synthesized in 1977 (Gilron and Flatters 2006; Yasaei et al. 2024), and was clinically developed as a structural analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), with GABA-mimetic effects and the ability to cross the blood-brain barrier (Di Cesare et al. 2023); moreover, GBP was approved for use as an antiepileptic drug (AED) in the United States for the first time in 1993 (NCBI 2024) by the FDA (Yassaei et al. 2024).

This drug is primarily intended for the treatment of epilepsy and peripheral neuropathic pain. Off-label, however, GBP is used in bipolar disorder and as prophylaxis for restless leg syndrome (Gilron and Flatters 2006; Abou-Khalil 2019; Brewer et al. 2022) or migraine (Magnus 1999; Mathew et al. 2001). However, the mechanism of action of GBP is still not fully understood. Several sources have reported that it acts as a selective blocker of voltage-gated calcium channels. In addition, GBP reduces exocytosis and neurotransmitter release from presynaptic terminals (Quintero 2017). In contrast, Eroglu et al. (2009) and Bauer et al. (2010) reported that GBP reduces excitatory synapse formation through action on the α2-δ1 subunit of calcium channels (Quintero 2017). In addition, complementary research by Marais et al. (2001) suggested that GBP has

Address for correspondence:
Mgr. Bc. Renáta Hesová
Department of Animal Protection and Welfare and Veterinary Public Health
Faculty of Veterinary Hygiene and Ecology
University of Veterinary Sciences Brno
Palackeho tr. 1946/1, Brno 612 42, Czech Republic
E-mail: hesovar@vfu.cz
http://actavet.vfu.cz/

a specific affinity for the α2-δ1 subunit, less affinity for the α2-δ2 subunit, and no affinity for the α2-δ3 subunit (Quintero 2017). However, some researchers still report that the mechanism of action of GBP is not yet fully understood, which adds to its complexity (Sills 2006; Dal Bello et al. 2020; Di Cesare et al. 2023).

Pharmacokinetic and metabolism studies of GBP were performed in rats and dogs after intravenous and intragastric administration of a single dose of [14C]-GBP (Prakash 2014). Vollmer et al. (1986) have reported that no biotransformation of GBP occurrs in humans. In rats, biotransformation is only slight. However, the remarkable formation of N-methyl-gabapentin has been observed in dogs. Radulovic et al. (1995) reported similar results and described the metabolism of GBP in monkeys. Except for that, in dogs, the metabolism of GBP in mice, rats, and monkeys was minimal (< 5%). In all species, GBP was almost exclusively excreted via the kidneys. Based on these studies, GBP was not metabolised and did not affect the induction or inhibition of liver metabolism. There was no plasma protein binding. Gabapentin has not been associated with drug-drug interactions. The elimination half-life in humans is between 5 and 6 h. After oral administration, the renal elimination rate was found to be ~80%. Lower doses and less frequent dosing may be used in patients with renal insufficiency (Prakash 2014). After administration, GBP is excreted from the human body and is completely unmetabolised (McLean 1994; Gallimore and Gidal 2010; Dal Bello et al. 2020).

Since wastewater treatment plants (WWTPs) are not completely effective at removing GBP, some bioactive molecules of GBP may be released unchanged into the environment (Dal Bello et al. 2020). WWTPs thus play a key role in controlling environmental contamination. Pollutants and micropollutants are removed from wastewater using traditional chemical, physical, and biological processes combined with advanced oxidation processes such as semiconductor-mediated photocatalysis (Dal Bello et al. 2020).

This review aimed to assess the potential environmental impact of GBP due to its overuse and nonprescription use in human and veterinary practice, focusing on its effects on the aquatic environment and organisms living in this habitat. This study included the assessment of factors such as the presence of the medicinal product in water and its toxicity to aquatic organisms.

General information on classification, uses, prescription, and side effects

Generally, there are several ways to categorise AEDs. The three most common classifications of AEDs are described. The first approach is to divide them into groups from a historical perspective, i.e., into so-called ‘old’ (Bialer 2006; Schmidt 2007) and ‘new’ AEDs (Duncan 2002). The ‘old’ (or also established) (Brodie and Dichter 1997; White 1999; Sander 2004; Lee and Dworetzky 2010) or essential (Corrales-Hernández et al. 2023) AEDs refer to drugs developed before 1993. This group is mainly represented by four substances: phenobarbital, phenytoin, carbamazepine, and valproate (Lee and Dworetzky 2010). However, these drugs have little or no effect on seizures in approximately one-third of patients, and some of them also have troublesome side effects, such as diplopia, nausea, psychomotor slowing, or hair loss (St. Louis 2009). The second group is represented by so-called ‘new’ antiepileptic drugs (Duncan 2002; Hirsch et al. 2003), which include substances such as GBP, pregabalin, lamotrigine, vigabatrin, levetiracetam, brivaracetam and others. While older drugs act against seizures primarily by blocking neuronal sodium channels (phenytoin and carbamazepine) or by increasing GABAergic inhibition (phenobarbital and valproate), some of the newer drugs have different mechanisms of action (Nakken and Brodtkorb 2020). By inhibiting the degradation of GABA, vigabatrin increases GABAergic inhibition at synapses. In the presynaptic boutons of neurons, levetiracetam and brivaracetam bind to the synaptic

vesicle protein SV2A, altering neurotransmitter release. Perampanel blocks the glutamate receptor α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, while lacosamide enhances the slow inactivation of sodium channels. Gabapentin, pregabalin, and, to some extent, zonisamide act as calcium channel blockers. However, several of the new drugs are minor structural modifications of older representatives (oxcarbazepine, eslicarbazepine, pregabalin, and brivaracetam). Like their predecessors, these new drugs treat symptoms only by preventing seizures and do not affect epilepsy itself (Kobayashi et al. 2020; Nakken and Brodtkorb 2020).

The second approach to determining how AEDs can be classifed is based on their mechanism of action (Davies 1995), which divides anticonvulsants into the following four groups: (1) modulating voltage-gated sodium, calcium or potassium channels (phenytoin, carbamazepine, lamotrigine, and valproate); (2) altering GABAergic inhibition via actions at GABAA receptors or GABA synthesis, reuptake or degradation (benzodiazepines, barbiturates, and tiagabine); (3) reducing synaptic excitation via actions at ionotropic glutamate receptors (phenobarbital and topiramate); and (4) modulating neurotransmitter release via presynaptic mechanisms, most importantly glutamate release (lamotrigine and felbamate) (Porter et al. 2012; Söderpalm 2012; Van Liefferinge et al. 2013). However, several of the above drugs fall into more than one or all categories (e.g., phenobarbital, topiramate, and valproate), and it is often unclear which is most important for a particular drug effect (Rogawski and Porter 1990; Söderpalm 2012). However, gabapentin does not fit neatly into these traditional categories. Its primary mechanism of action involves binding to the α2δ subunit of voltage-gated calcium channels, thereby reducing the presynaptic release of excitatory neurotransmitters such as glutamate and norepinephrine (Van Liefferinge et al. 2013). It is therefore generally classified as a calcium channel modulator, distinct from classical AEDs that target sodium channels or GABA receptors.

Third, AEDs can be classified according to their chemical structure using the Anatomical Therapeutic Chemical Classification of Drugs (ATC-classification), which defines the international system for classifying drugs that are maintained by the World Health Organisation Collaborating Centre for Drug Statistics Methodology (2023).

Gabapentin is a low-potency antiepileptic drug with a structure very similar to that of GABA; in fact, GBP was developed as a lipophilic, blood-brain barrier-permeable form of GABA. However, the drug does not interact with GABA systems. Gabapentin and pregabalin (collectively called 'gabapentinoids') have variable activities against tonic seizures in chemoconvulsive models (Söderpalm 2012).

According to the ATC classification, GBP, together with pregabalin, was recently removed from the group of patients receiving antiepileptics to the group receiving analgesics. This happened during the 51st Meeting of the World Health Organisation International Working Group for Drug Statistics Methodology in Geneva in 2022. This revision of the classification of the gabapentinoids was discussed earlier at the 46th meeting of the Working Group. Gabapentin and pregabalin were classified as N03AX agents or other antiepileptics, whereas mirogabalin, which is used for neuropathic pain, was classified as an N02BG, as other analgesics and antipyretics. Members considered that the main therapeutic use of GBP and pregabalin in recent years has been for the treatment of neuropathic pain. At the 51st meeting, the Working Group decided to create a new 4th-level ATC, N02BF Gabapentinoids, and to amend the classification of GBP, pregabalin, and mirogabalin (World Health Organisation 2022).

The analgesic effects of GBP were discovered in the mid-1990s, but its exact mode of action is still discussed. It was originally thought to act as an analogue of the inhibitory neurotransmitter GABA, where it readily crosses the blood-brain barrier and precisely imitates the effects of GABA. However, GBP does not bind to GABA receptors

(Gilron and Flatters 2006; Herrmann et al. 2015). The analgesic effects are attributed to binding to the α2δ subunit of voltage-dependent calcium channels (Nicholson 2000), reducing the release of excitatory neurotransmitters such as noradrenaline and dopamine. Moreover, the inhibition of glutamate synthesis, increased serotonin levels, and competition with membrane L-amino acid transport are often implicated.

Apart from epilepsy and neuropathic pain, it is often used in bipolar disorder and as prophylaxis for restless legs syndrome (Gilron and Flatters 2006; Abou-Khalil 2019; Brewer et al. 2022) or migraine (Magnus 1999; Mathew et al. 2001). The official PubChem website of the National Center for Biotechnology Information (NCBI) states that GBP is approved for the treatment of postherpetic neuralgia in adults and the adjunctive treatment of partial-onset seizures, with or without secondary generalisation, in patients aged 3 years and older in the U.S. In Europe, GBP is indicated as adjunctive therapy for the treatment of partial-onset seizures with or without secondary generalisation in patients 6 years of age and older and as monotherapy in patients 12 years of age and older. It is also used in adults to treat various types of peripheral neuropathic pain, such as painful diabetic neuropathy. Its popularity in medicine is related to its distinct advantages over other AEDs, such as a relatively benign side effect profile, a broad therapeutic index, and a lack of significant metabolism, making it unlikely to be involved in pharmacokinetic drug interactions (NCBI 2024).

The adverse side effects of GBP tend to be related to the central nervous system. These conditions are mild to moderate in severity and occur mainly in the first 2–3 weeks of treatment (McLean 1995; Somerville et al. 2015). Morris (1999) and Magnus (1999) list the most common adverse reactions as drowsiness, dizziness, and ataxia. Weight gain has sometimes been reported with higher doses of GBP, and paediatric reports cite marked behavioural changes, including hyperactivity, irritability, and agitation.

The increasing use of gabapentinoids (including GBP and pregabalin) in various countries has raised concerns in recent studies. Chan et al. (2023) reported the consumption of gabapentinoids in 65 countries and regions from 2008 to 2018. This longitudinal trend study assessed global trends in gabapentinoid consumption using pharmaceutical sales data from 65 countries and regions worldwide. The multinational average annual percentage change in gabapentinoid consumption was +17.20%. It increased from 4.17 defined daily doses per ten thousand inhabitants per day (DDD/TID) in 2008 to 18.26 DDD/TID in 2018. The highest average annual increase in consumption was observed in North Africa, followed by East Asia, Eastern Europe, and Central Asia. High-income countries (e.g., Bahrain, Canada, Norway, Saudi Arabia, United States) had the highest gabapentinoid consumption rate in 2018 (39.92 DDD/TID). This number was more than six times greater than that in lower-middle-income countries, e.g., Cambodia, Kenya, Nepal, and Vietnam (6.11 DDD/TID). In 2018, the pooled gabapentinoid consumption was highest in North America (124.62 DDD/TID; 95% CI, 95.77 to 162.16), followed by Oceania (68.88 DDD/TID; 95% CI, 37.14 to 127.72), and Northern Europe (54.66 DDD/TID; 95% CI, 38.59 to 77.43). Gabapentinoid consumption was lowest in Central Asia (1.05 DDD/TID; 95% CI, 1.04 to 1.05) (Chan et al. 2023).

Gabapentin in veterinary practice

The veterinary use of GBP has increased dramatically in recent years, likely because of its use as an oral analgesic alternative to nonsteroidal anti-inflammatory drugs and because of the lack of evidence for the analgesic effects of tramadol in some species, especially dogs (Reader et al. 2021). Gabapentin was originally used off-label as an analgesic (Reader et al. 2021) and an antiepileptic in dogs and cats, but it is now more commonly used to treat chronic and neuropathic pain in companion animals, despite the

lack of evidence for its efficacy and the lack of correspondence between neuropathic pain conditions in humans and animals (Di Cesare et al. 2023). However, in dogs, GBP has shown beneficial effects in the treatment of epilepsy (Govendir et al. 2005; Platt et al. 2006). In this case, GBP is used in combination with commonly used AEDs such as phenobarbitone and/or potassium bromide. Govendir et al. (2005) reported that the short half-life of GBP has advantages in terms of seizure control, but the current high cost of GBP may preclude its use in large dogs. Moreover, mild side effects, such as ataxia and sedation, may be observed in dogs but are not severe enough to require discontinuation (Platt et al. 2006). In addition to its use in treating epilepsy, GBP is also used in dogs to treat chronic and neuropathic pain (Ruel et al. 2020; Di Cesare et al. 2023) and anxiety (Bleuer-Elsner et al. 2021; Meneses et al. 2021; Di Cesare et al. 2023). In cats, this technique has shown efficacy in the management of post-ovariohysterectomy pain and anxiety (Di Cesare et al. 2023). Several studies on anxiety, which has recently become an increasingly discussed problem in dogs and cats, have evaluated the use of GBP in cats (van Haaften et al. 2017; Pankratz et al. 2018; Hudec and Griffin 2020; Meneses et al. 2021) but not in dogs. In horses, GBP has been used as an analgesic to treat chronic pain (conditions that are associated with neuropathic and chronic pain include laminitis, arthritis, idiopathic head shaking, and navicular syndrome), but the results are variable and suggest that further studies are needed (Di Cesare et al. 2023). Davis et al. (2007) treated a 24-year-old, 732 kg pregnant Belgian draft horse mare that presented symptoms of neuropathy and intractable pain. Shortly after starting GBP treatment, the mare appeared well and had no further signs of pain. The mare subsequently gave birth to a healthy foal. This was the first use of GBP in horses. In chronic pain, GBP was ineffective as a monotherapy for chronic lameness and did not improve subjective or objective measures of lameness in horses with chronic thoracic musculoskeletal pain when used alone, but did reduce pain when combined with firocoxib. Moreover, it has been shown to have no adverse effects, i.e., cardiovascular, sedative, or behavioural effects or changes in physiological or biochemical variables, on healthy horses (Di Cesare et al. 2023).

Occurrence of gabapentin in aquatic environments

As mentioned in the introduction, unmetabolised GBP enters wastewater and, consequently, surface water (Dal Bello 2020). Numerous studies have confirmed the widespread presence of GBP in aquatic environments across different countries. Concentrations of GBP in influent wastewater have been reported to range from a few micrograms per litre (µg/l) (e.g. 13.2 ± 3.3 µg/l; Gurke et al. 2015) to 54 µg/l (Vymazal et al. 2017), while effluent concentrations can vary significantly depending on the wastewater treatment technology used. Some studies have reported values as low as 2.6 µg/l (Kasprzyk-Hordern et al. 2009). Surface water concentrations range from tens to hundreds of nanograms per litre (ng/l). For example, concentrations of 304 ng/l of GBP were found in Korean rivers and 180 ng/l in drinking water (Ra et al. 2020), while concentrations of up to 353 ng/l were reported in Czech rivers (Ferencik et al. 2022). An overview of GBP concentrations in various matrices is provided in Table 1. The data presented illustrate variability in environmental GBP levels and removal efficiencies.

The efficiency of GBP removal in conventional WWTPs varies greatly depending on treatment design and operational conditions. Activated sludge systems tend to show higher removal rates (up to 84%; Kasprzyk-Hordern et al. 2009) compared to trickling filters or constructed wetlands, where removal can be below 15% (Vymazal et al. 2017). Nevertheless, removal efficiency is influenced by several factors such as hydraulic retention time, temperature, microbial composition, and presence of heterotrophic degraders (Margot et al. 2016).

Table 1. Overview of reported gabapentin (GBP) concentrations in wastewater and surface waters across different geographic regions.

Study / Year	Location / Matrix	Type	GBP concentration (µg/l)	Notes
Gurke et al. (2015)	Germany / WWTP	Influent / Effluent	13.20 ± 3.30 / 12.10 ± 2.60	High load entering WWTP
Kasprzyk-Hordern et al. (2008)	UK / Rivers Taff & Ely	Surface water	0.10-0.97	Upstream-downstream WWTP gradient
Kasprzyk-Hordern et al. (2009)	UK / Coslech WWTP	Influent /Effluent	17.90 / 2.60	Trickling filter treatment
Writer et al. (2013)	USA / WWTP & surface water	Sludge / pond	0.56-1.20 ± 0.94	88% detection frequency
Vymazal et al. (2017)	CZ / Constructed wetlands	Influent	8.01-54.00	Removal efficiency ~14%
Ferencik et al. (2022)	CZ / Elbe River basin	Surface water	0.08-0.35	Varies with proximity to urban sources
Ra et al. (2020)	Korea / River & drinking water	Effluent / drinking	1.29 / 0.18	GBP-nitrile formation noted
Goswami et al. (2019)	Germany / Lake Constance	Surface water	0.04	Used to test ozonation efficiency

Concentrations are presented as means or ranges, with units in µg/l as reported in the original studies. WWTP – Waste water treatment plant; UK – United Kingdom of Great Britain and Northern Ireland; USA – United States of America; CZ – Czech Republic

A further concern is the formation of transformation products during water treatment. Ra et al. (2020) documented the transformation of GBP into 1-cyanocyclohexyl acetic acid (GBP-nitrile) under chlorination. While its acute toxicity is considered low, the long-term ecological impacts and potential for bioaccumulation are not yet fully understood. Similarly, other oxidative processes (e.g., ozonation) may yield unknown intermediates that could persist or exert toxic effects.

Advanced treatment technologies, including ozonation, activated carbon adsorption, and membrane filtration, have been tested for GBP removal. Goswami et al. (2019) demonstrated that ozonation at 0.9 mg/l removed 88% of GBP after 20 min, reaching nearly 100% after 45 min. Margot et al. (2013) reported that conventional treatment yielded only 9.2% removal, whereas the addition of powdered activated carbon and ultrafiltration increased removal to 11.8%, and ozonation up to 38%. Reverse osmosis and biologically activated carbon systems may offer additional removal efficiency but remain costly for widespread implementation.

Despite these efforts, the frequency of GBP detection remains high. It was found in 88% of wastewater samples (Writer et al. 2013) and in over 90% of samples from Czech constructed wetlands (Vymazal et al. 2017). Concentrations in surface waters vary from 40 ng/l in Lake Constance (Goswami et al. 2019) to over 350 ng/l in areas downstream of urban centres (Ferencik et al. 2022). In Korea, GBP was detected in river water at 304 ng/l and even in drinking water at 180 ng/l (Ra et al. 2020), confirming its persistence and mobility through water treatment chains.

In summary, GBP is an environmentally persistent compound whose removal via conventional wastewater treatment is inconsistent and often incomplete. Its transformation into secondary metabolites, some with unknown toxicological profiles, further complicates environmental risk assessments. Future studies should prioritise the long-term fate and ecotoxicity of both parent and transformation compounds, as well as optimise removal strategies that are both effective and economically feasible.

Effects of gabapentin on aquatic organisms

In addition to the proven teratogenicity of GBP in mice, evaluating the effect of GBP on aquatic organisms is important due to its persistence in the aquatic environment. Although acute toxicity data suggest low immediate lethality, there is increasing evidence that it can induce subtle yet significant sublethal effects in a variety of aquatic species, even at concentrations relevant to the environment. In a recent review, Salahinejad et al. (2023) summarised the known effects of various antiepileptic drugs, including gabapentin, on teleost fish. Their analysis revealed consistent effects, including oxidative stress, locomotion disruption, behavioural alterations and endocrine interference, particularly with chronic exposure. These mechanisms may impact the fitness of individuals and, in the long term, the dynamics of fish populations in exposed communities.

Li et al. (2018) reported the effect of GBP on the early development of zebrafish (*Danio rerio*) and its antioxidant system. Acute toxicity tests revealed that the 50% lethal concentration of GBP at 96 h post fertilisation (hpf) was 59.9 g/l. The presence of malformations such as haemagglutination and pericardial oedema was noted. Compared to that in the control group, the heartbeat rate was significantly higher ($P < 0.05$) at concentrations exceeding 50 mg/l, and the swimming frequency was increased after exposure to a concentration of 100 mg/l ($P < 0.05$). In addition, embryonic development was negatively affected, as demonstrated by a significantly reduced body length. Exposure to GBP at concentrations exceeding 50 mg/l resulted in organ malformation and abnormal movements. Although no significant effects on embryonic development were observed at environmentally relevant concentrations (0.1 and 10 µg/l), further investigation of the antioxidant system confirmed that severe internal oxidant damage occurred. The results showed increased activity of catalase, lactate dehydrogenase, glutathione S-transferase, and glutathione, as well as increased amounts of hydroxyl radicals. Among the biomarkers, catalase was the most sensitive at assessing the effect of GBP, as it exhibited a significant increase in activity even at very low exposure concentrations (0.1 µg/l) (Li et al. 2018).

Similarly, He et al. (2019) studied the effect of GBP on embryos of zebrafish. In the test, specimens were exposed to environmental concentrations of 0.1 and 10 µg/l. To determine the underlying mechanisms involved, transcriptomic profiling via deep sequencing was performed. The embryos were exposed to GBP from 12 to 96 hpf. The results of gene ontology analysis and the Kyoto Encyclopaedia of Genes and Genomes pathway analysis illustrated that many differentially expressed genes were involved in the antioxidant, immune, and nervous systems. Moreover, reduced acetylcholinesterase activity, lysozyme activity, and reduced C-reactive protein contents were observed at the end of exposure, which correlated with the transcriptomic data. Thus, this study demonstrated that GBP simultaneously affects various vital developmental functions in the early developmental stage of zebrafish, even at environmentally relevant concentrations (He et al. 2019).

In addition to the above study, He et al. (2024) conducted an extension study in which zebrafish embryos were exposed to GBP at environmentally relevant concentrations (0, 0.1, 10 and 1000 µg/l) to assess its effect on the cardiovascular system during the early life stages of zebrafish. GBP exposure was found to increase heart rate and blood flow. Vascular development was also affected, with a significant decrease in vessel width observed at concentrations of 10 µg/l and above. At the same time, GBP exposure led to abnormal vessel development by inhibiting the expression of relevant genes (flk1, vegfr-3, gata1, vegfα and vegfr-2). Furthermore, GBP at a concentration of 0.1 µg/l increased reactive oxygen species and antioxidant enzyme levels. However, these adverse effects were reversible with the antioxidant N-acetyl-L-cysteine, which highlights the key role of oxidative damage in GBP-induced vascular toxicity (He et al. 2024).

A year later, He et al. (2025a) investigated the effects of GBP (at concentrations of 0.1, 10 and 1000 µg/l) on the visual development of zebrafish. Behavioural tests revealed that exposure to GBP increased light sensitivity, as demonstrated by a notable rise in total travel distance (TTD) across all exposure groups compared to the control groups. Groups exposed to concentrations of 1 µg/l and 1,000 µg/l showed increases in TTD of 41% and 37%, respectively *(P* < 0.05). Apoptotic tests revealed dose-dependent retinal cell death, with fluorescence intensity increasing by 15% at 1,000 µg/l *(P* < 0.05). The results of this study demonstrate, among other things, that GBP disrupts vision development in zebrafish through retinal apoptosis and thyroid hormone dysregulation, thereby highlighting the ecological risks posed by pharmaceutical pollutants. Gabapentin exposure increased light-induced locomotor activity, suggesting increased sensitivity to light due to retinal apoptosis. Even at concentrations as low as 1 µg/l, exposure to GBP led to a significant reduction in the optokinetic response to different colours, likely due to changes in retinal thickness associated with thyroid dysfunction. These effects were consistent with changes in gene expression related to apoptosis, thyroid function and retinal development (He et al. 2025b).

Similar results were obtained by Blahova et al. (2025), who investigated the sublethal effects of GBP on the common carp (*Cyprinus carpio*). Following exposure to environmentally relevant concentrations, they reported altered antioxidant and biochemical responses, supporting the hypothesis that oxidative imbalance is a key mechanism of GBP toxicity in aquatic vertebrates (Blahova et al. 2025)

Henry et al. (2022) demonstrated the use of a specially developed high-performance multidimensional battery of behavioural tests on zebrafish larvae at 5 dpf. The automated battery consisted of established tests of spontaneous swimming, simulated predator response, photomotor response of larvae, and a new thermotaxis test. This system was used to characterise environmentally relevant concentrations of new pharmaceutical micropollutants, including gabapentin at a concentration of 400 ng/l. In this study, it was found that GBP affects photomotor responses in larval stages of fish (Henry et al. 2022).

O'Rourke et al. (2023a) recently conducted a pilot study in which they investigated the effect of GBP on daphnids as a model species with significant characteristics for ecology and ecotoxicology. By combining multiple endpoints, such as mortality, biochemical (enzyme activity), and holistic (metabolomic) data, distinct patterns of biological responses were identified. In this study, changes in metabolic enzymes such as phosphatases and lipase, as well as the detoxification enzyme glutathione-S-transferase, were recorded after acute exposure to GBP at a concentration of 50 mg/l. Daphnids less than 24 h old were cultured until they reached 4 days of age and were subsequently exposed to the relevant pharmaceuticals and concentrations for 24 h. As a result, GBP does not affect mortality. Nevertheless, concerning the metabolomics data, it was found that, compared with those in the controls, the metabolic phenotypes of daphnids exposed to gabapentin, a freely water-soluble drug, significantly changed. Nicotinamide and thiamine were found to be decreased in daphnids treated with GBP. Overall, based on the significantly affected metabolites, water-soluble drugs (including GBP) significantly dysregulate the metabolic pathways of daphnids related to energy and carbohydrate metabolism, amino acid metabolism, purine metabolism, and pyrimidine metabolism (O'Rourke et al. 2023a).

O'Rourke et al. (2023b) assessed the effects of GBP on daphnids by monitoring physiological markers such as enzyme activities combined with metabolic disturbances. The physiological marker activity of phosphatase, lipase, peptidase, β-galactosidase, lactate dehydrogenase, glutathione S-transferase, and glutathione reductase was assessed. Targeted liquid chromatography-tandem mass spectrometry analysis was also performed to assess metabolic changes. This analysis focused on glycolysis, the pentose phosphate pathway, and tricarboxylic acid cycle intermediates. In the end, GBP was responsible for

most of the differences in the contents of metabolites, and these changes were associated with changes in the activities of several enzymes, such as decreasing acid phosphatase activities and increasing lipase, lactate dehydrogenase, and glutathione-S-transferase activity. For glycolysis, glucose-6-phosphate was decreased in GBP experiments, but a shift towards the pentose phosphate pathway was observed in exposures, as deduced from the increase in ribulose-5-phosphate and ribose-5-phosphate (O'Rourke et al. 2023b).

While the majority of studies to date have centred on fish and invertebrates, Salahinejad et al. (2023) also emphasised the potential impact of antiepileptic compounds, including GBP, on primary producers. These include oxidative stress, inhibited growth and altered pigment composition in algal and cyanobacterial species. However, direct evidence for the impact of GBP remains limited, and further research is needed to assess its ecotoxicological effects on the fundamental components of aquatic ecosystems (Salahinejad et al. 2023).

Notably, none of these studies have addressed the potential toxicity of GBP degradation products. This is a cause for concern, given that many wastewater treatment plants are now adopting advanced treatment technologies such as ozonation or UV irradiation, which can generate transformation products with unknown biological effects. For example, Pohl et al. (2020) demonstrated that ozonation of carbamazepine (another antiepileptic drug) led to the formation of transformation products that were equally or more toxic to zebrafish embryos. This highlights the need to evaluate whether similar GBP degradation products might pose additional ecological risks (Pohl et al. 2020).

In summary, GBP is not acutely toxic to aquatic organisms at concentrations currently detected in surface waters. However, it can affect antioxidant defence mechanisms, immune function, neurodevelopment and central metabolism at environmentally relevant concentrations. These findings highlight the importance of considering sublethal endpoints and long-term ecological effects in future risk assessments of GBP and related pharmaceuticals.

Conclusion

Gabapentin is a widely used therapeutic agent. Its increasing use in human and veterinary medicine has raised environmental concerns due to its excretion in an unmetabolised form, and its incomplete removal by conventional wastewater treatment processes. Although GBP exhibits low acute toxicity to aquatic organisms, numerous studies have demonstrated sublethal effects, particularly at environmentally relevant concentrations in the ng/l to µg/l range. These include oxidative stress, altered gene expression, cardiovascular and developmental toxicity, and disruption of neuroimmune functions in species such as zebrafish and daphnids. Gabapentin has also been shown to interfere with metabolic pathways and induce apoptosis, highlighting its potential to affect individual fitness and population-level processes. While some WWTPs achieve partial removal of GBP, treatment efficiency varies significantly, and advanced processes such as ozonation can produce transformation products whose ecological risks are largely unknown. Importantly, the toxicological profiles of these by-products, such as GBP-lactam and GBP-nitrile, require further investigation. In light of the growing body of evidence regarding the biological effects of GBPs, there is an urgent need for enhanced monitoring, targeted ecotoxicological studies and more efficient removal technologies. This review emphasises the dual role of GBP as a valuable pharmaceutical and an emerging environmental contaminant, calling for integrated management approaches that address its environmental footprint while maintaining its therapeutic utility.

Acknowledgements

This work was supported by the Internal Grant Agency of the Veterinary University Brno, project number 219/2023/FVHE.

References

Abou-Khalil BW 2019: Update on antiepileptic drugs 2019: Continuum (Minneap Minn) **25**: 508-536

Bauer CS, Tran-van-Minh A, Kadurin I, Dolphin AC 2010: A new look at calcium channel α2δ subunits. Curr Opin Neurobiol **20**: 563-571

Bialer M 2006: New antiepileptic drugs that are second-generation to existing antiepileptic drugs. Expert Opin Investig Drugs **15**: 637-647

Blahova J, Mikula P, Marsalek P, Svobodova Z 2025: Biochemical and antioxidant responses of common carp (*Cyprinus carpio*) exposed to sublethal concentrations of the antiepileptic and analgesic drug gabapentin. Vet Med **70**: 20-29

Bleuer-Elsner S, Medam T, Masson S 2021: Effects of a single oral dose of gabapentin on storm phobia in dogs: A double-blind, placebo-controlled crossover trial. Vet Rec **189**: e453

Brewer K, Machin J, Maylin G, Fenger C, Morales-Briceño A, Tobin T 2022: Gabapentin, a human therapeutic medication an environmental substance transferring at trace levels to horses: a case report. Ir Vet J **75**: 19

Brodie MJ, Dichter MA 1997: Established antiepileptic drugs. Seizure **6**: 159-174

Chan AYL, Yuen ASC, Tsai DHT et al. 2023: Gabapentinoid consumption in 65 countries and regions from 2008 to 2018: a longitudinal trend study. Nat Commun **14**: 5005

Corrales-Hernández M, Villarroel-Hagemann S, Mendoza-Rodelo I, Palacios-Sánchez L, Gaviria-Carrillo M, Buitrago-Ricaurte N, Espinosa-Lugo S, Calderon-Ospina CA, Rodríguez-Quintana JH 2023: Development of antiepileptic drugs throughout history: From serendipity to artificial intelligence. Biomedicines **11**: 1632

Dal Bello F, Medina C, Zorzi M, Kuck B, Fabri D, Calza P 2020: Liquid chromatography/mass spectrometry analytical determination of gabapentin transformation products by heterogeneous photocatalysis and environmental evaluation. Rapid Commun Mass Spectrom **34**: e8925

Davies JA 1995: Mechanisms of action of antiepileptic drugs. Seizure **4**: 267-271

Davis JL, Posner LP, Elce Y 2007: Gabapentin for the treatment of neuropathic pain in a pregnant horse. J Am Vet Med Assoc **231**: 755-758

Di Cesare F, Negro V, Ravasio G, Villa R, Draghi S, Cagnardi P 2023: Gabapentin: clinical use and pharmacokinetics in dogs, cats, and horses. Animals (Basel) **13**: 2045

Duncan JS 2002: The promise of new antiepileptic drugs. Br J Clin Pharmacol **53**: 123-131

Eroglu Ç, Allen NJ, Susman MW, O'Rourke NA, Park CY, Ozkan E, Chakraborty C, Mulinyawe SB, Annis DS, Huberman AD, Green EM, Lawler J, Dolmetsch R, Garcia KC, Smith SJ, Luo ZD, Rosenthal A, Mosher DF, Barres BA 2009: Gabapentin receptor α2δ-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. Cell **139**: 380-392

Ferencik M, Blahova J, Schovankova J, Siroka Z, Svobodova Z, Kodes V, Stepankova K, Lakdawala P 2022: Residues of selected anticonvulsive drugs in surface waters of the Elbe River Basin (Czech Republic). Water (Basel) **14**: 4122

Gallimore C, Gidal B 2010: Gabapentin. In: Panayiotopoulos CP (Ed.): Atlas of Epilepsies. Springer, London, pp. 1753-1760

Gilron I, Flatters S 2006: Gabapentin and pregabalin for the treatment of neuropathic pain: A review of laboratory and clinical evidence. Pain Res Manag **11**: 16A-29A

Goswami A, Jiang JQ, Petri M 2019: Non-parametric regression analysis of diuron and gabapentin degradation in Lake Constance water by ozonation and their toxicity assessment. Water (Basel) **11**: 852

Govendir M, Perkins M, Malik R 2005: Improving seizure control in dogs with refractory epilepsy using gabapentin as an adjunctive agent. Aust Vet J **83**: 602-608

Gurke R, Rössler M, Marx C, Diamond S, Schubert S, Oertel R, Fauler J 2015: Occurrence and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater of a sewage treatment plant. Sci Total Environ **532**: 762-770

He Y, Hu J, Freitas R, Gu J, Ji G, Zhang Y 2024: Trace-level gabapentin can induce cardiovascular developmental toxicity through apoptosis in zebrafish larvae. Environ Pollut **358**: 124526

He Y, Li X, Jia D, Zhang W, Zhang T, Yu Y, Xu Y, Zhang Y 2019: A transcriptomics-based analysis of the toxicity mechanisms of gabapentin to zebrafish embryos at realistic environmental concentrations. Environ Pollut **251**: 746-755

He Y, Li X, Yang Y, Freitas R, Zhu J, Ji G, Zhang Y 2025a: Gabapentin impairs visual development in zebrafish via retinal apoptosis and thyroid disruption. J Hazard Mater **487**: 137299

He Y, Wang C, Sun Q, Liu R, Wang C, Zhou R, Freitas R, Zhang Y 2025b: Are environmental levels of gabapentin (GBP) a cause for concern? Chronic reproductive effects of GBP in zebrafish. J Hazard Mater **492**: 138173

Henry J, Bai Y, Kreuder F, Saaristo M, Kaslin J, Wlodkowic D 2022: Sensory-motor perturbations in larval zebrafish (*Danio rerio*) induced by exposure to low levels of neuroactive micropollutants during development. Int J Mol Sci **23**: 8990

Herrmann M, Menz J, Olsson O, Kümmerer K 2015: Identification of phototransformation products of the antiepileptic drug gabapentin: Biodegradability and initial assessment of toxicity. Water Res **85**: 11-21

Hirsch E, Schmitz B, Carreño M 2003: Epilepsy, antiepileptic drugs (AEDs) and cognition. Acta Neurol Scand **108**: 23-32

Hudec CP, Griffin CE 2020: Changes in the stress markers cortisol and glucose before and during intradermal testing in cats after a single administration of pre-appointment gabapentin. J Feline Med Surg **22**: 138-145
Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ 2008: The occurrence of pharmaceuticals, personal care products, endocrine disruptors, and illicit drugs in surface water in South Wales, UK. Water Res **42**: 3498-3518
Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ 2009: The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res **43**: 363-380
Kobayashi K, Endoh F, Ohmori I, Akiyama T 2020: Action of antiepileptic drugs on neurons. Brain Dev **42**: 2-5
Lee JW, Dworetzky B 2010: Rational polytherapy with antiepileptic drugs. Pharmaceuticals **3**: 2362-2379
Li X, Zhou S, Qian Y, Xu Z, Yu Y, Xu Y, He Y, Zhang Y 2018: The assessment of the eco-toxicological effect of gabapentin on early development of zebrafish and its antioxidant system. RSC Adv **8**: 22777-22784
Macdonald RL, Kelly KM 1995: Antiepileptic drug mechanisms of action. Epilepsia **36**: S2-S12
Magnus L 1999: Nonepileptic uses of gabapentin. Epilepsia **40**: S66-S72
Mao J, Chen LL 2000: Gabapentin in pain management. Anesth Analg **91**: 680-687
Marais E, Klugbauer N, Hofmann F 2001: Calcium channel α2δ subunits—structure and gabapentin binding. Mol Pharmacol **59**: 1243-1248
Margot J, Kienle C, Magnet A, Weil M, Rossi L, De Alencastro LF, Abegglen C, Thonney D, Chèvre N, Schärer M, Barry D 2013: Treatment of micropollutants in municipal wastewater: Ozone or powdered activated carbon? Sci Total Environ **461-462**: 480-498
Margot J, Lochmatter S, Barry DA, Holliger CH 2016: Role of ammonia-oxidizing bacteria in micropollutant removal from wastewater with aerobic granular sludge. Water Sci Technol **73**: 564-575
Mathew NT, Rapoport A, Saper J, Magnus L, Klapper J, Ramadan N, Stacey B, Tepper S 2001: Efficacy of gabapentin in migraine prophylaxis. Headache **41**: 119-128
McLean MJ 1994: Clinical pharmacokinetics of gabapentin. Neurology **44**: 17-22
McLean MJ 1995: Gabapentin. Epilepsia **36**: S73-S86
Meneses T, Robinson J, Rose J, Vernick J, Overall KL 2021: Development of and pharmacological treatment options and future research opportunities for separation anxiety in dogs. J Am Vet Med Assoc **259**: 1130-1139
Morris GL 1999: Gabapentin. Epilepsia **40**: S63-S70
Nakken KO, Brodtkorb E 2020: Are the new anti-epileptic drugs any better than their predecessors? Tidsskr Nor Laegeforen **140**: 1-7
National Center for Biotechnology Information (NCBI) 2024: PubChem Compound Summary for CID 3446, Gabapentin. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Gabapentin. Accessed March 5, 2025
Nicholson B 2000: Gabapentin use in neuropathic pain syndromes. Acta Neurol Scand **101**: 359-371
O'Rourke K, Virgiliou CH, Theodoridis G, Gika H, Grintzalis K 2023a: The impact of pharmaceutical pollutants on daphnids – A metabolomic approach. Environ Toxicol Pharmacol **100**: 104157
O'Rourke K, Engelmann B, Altenburger R, Rolle-Kampczyk U, Grintzalis K 2023b: Molecular responses of daphnids to chronic exposures to pharmaceuticals. Int J Mol Sci **24**: 4100
Pankratz KE, Ferris K, Griffith E, Sherman BL 2018: Use of single-dose oral gabapentin to attenuate fear responses in cage-trap confined community cats: a double-blind, placebo-controlled field trial. J Feline Med Surg **20**: 535-543
Platt SR, Adams V, Garosi L, Abramson CJ, Penderis J, De Stefani A, Matiasek L 2006: Treatment with gabapentin of 11 dogs with refractory idiopathic epilepsy. Vet Rec **159**: 881-884
Pohl J, Golovko O, Carlsson G, Eriksson J, Glynn A, Örn S et al. 2020: Carbamazepine ozonation byproducts: toxicity in zebrafish (*Danio rerio*) embryos and chemical stability. Environ Sci Technol **54**: 2913-2921
Porter RJ, Dhir A, Macdonald RL, Rogawski MA 2012: Mechanisms of action of antiseizure drugs. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV (Eds): Epilepsy. Handbook of Clinical Neurology. Elsevier, Volume 108, pp. 663-681
Prakash C 2014: Gabapentin. In: Lee PW (Ed.): Handbook of Metabolic Pathways of Xenobiotics. Wiley, Chichester.
Quintero GC 2017: Review about gabapentin misuse, interactions, contraindications and side effects. J Exp Pharmacol **9**: 13-21
Ra J, Yoom H, Son H, Lee Y 2020: Occurrence and transformation of gabapentin in urban water quality engineering: Rapid formation of nitrile from amine during drinking water chlorination. Water Res **184**: 116123
Radulovic LL, Türck D, von Hodenberg A, Vollmer KO, McNally WP, DeHart PD, Hanson BJ, Bockbrader HN, Chang T 1995: Disposition of gabapentin (neurontin) in mice, rats, dogs, and monkeys. Drug Metab Dispos **23**: 441-448
Reader R, Olaitan O, McCobb E 2021: Evaluation of prescribing practices for gabapentin as an analgesic among veterinary professionals. Vet Anaesth Analg **48**: 775-781
Rogawski MA, Porter RJ 1990: Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. Pharmacol Rev **42**: 223-286
Ruel HLM, Watanabe R, Evangelista MC, Beauchamp G, Auger JP, Segura M, Steagall PV 2020: Pain burden, sensory profile and inflammatory cytokines of dogs with naturally-occurring neuropathic pain treated with gabapentin alone or with meloxicam. PLoS One **15**: e0237121

Salahinejad A, Meuthen D, Attaran A, Chivers DP, Ferrari MCO 2023: Effects of common antiepileptic drugs on teleost fishes. Sci Total Environ **866**: 161324
Sander JW 2004: The use of antiepileptic drugs—principles and practice. Epilepsia **45**: 28-34
Schmidt B 2007: Clinical development of antiepileptic drugs in adults. Neurotherapeutics **4**: 62-69
Sills G 2006: The mechanisms of action of gabapentin and pregabalin. Curr Opin Pharmacol **6**: 108-113
Söderpalm B 2012: Anticonvulsants: aspects of their mechanisms of action. Eur J Pain **6**: 3-9
Somerville ER, Michell AW, Sen A 2015: Gabapentin. In: Shorvon S, Perucca E, Engel J (Eds): The Treatment of Epilepsy. 4th edition. Wiley, pp. 479-488
St Louis EK 2009: Minimizing AED adverse effects: improving quality of life in the interictal state in epilepsy care. Curr Neuropharmacol **7**: 106-114
van Haaften KA, Forsythe LRE, Stelow EA, Bain MJ 2017: Effects of a single preappointment dose of gabapentin on signs of stress in cats during transportation and veterinary examination. J Am Vet Med Assoc **251**: 1175-1181
Van Liefferinge J, Massie A, Portelli J, Di Giovanni G, Smolders I 2013: Are vesicular neurotransmitter transporters potential treatment targets for temporal lobe epilepsy? Front Cell Neurosci **7**: 1-10
Vollmer KO, von Hodenberg A, Kölle EU 1986: Pharmacokinetics and metabolism of gabapentin in rat, dog and man. Arzneimittelforschung **36**: 830-839
Vymazal J, Dvořáková Březinová T, Koželuh M, Kule L 2017: Occurrence and removal of pharmaceuticals in four full-scale constructed wetlands in the Czech Republic – the first year of monitoring. Ecol Eng **98**: 354-364
White HS 1999: Comparative anticonvulsant and mechanistic profile of the established and newer antiepileptic drugs. Epilepsia **40**: S2-S10
World Health Organization 2022: Executive Summary. 51st Meeting of the WHO International Working Group for Drug Statistics Methodology, March 24–25, 2022. Available at: https://cdn.who.int/media/docs/default-source/international-nonproprietary-names-(inn)/atc-ddd/51st_atc_ddd_executive_summary.pdf. Accessed January 7, 2025
World Health Organization Collaborating Centre for Drug Statistics Methodology 2023: ATC/DDD. Available at: https://www.whocc.no/atc_ddd_index/. Accessed January 23, 2025
Writer JH, Ferrer I, Barber LB, Thurman EM 2013: Widespread occurrence of neuro-active pharmaceuticals and metabolites in 24 Minnesota rivers and wastewaters. Sci Total Environ **461-462**: 519-527
Yasaei R, Katta S, Saadabadi A 2024: Gabapentin. In: StatPearls. StatPearls Publishing, Treasure Island (FL). Available at: https://www.ncbi.nlm.nih.gov/books/NBK493228/. Accessed March 5, 2025