

RESEARCH ARTICLE

Bilastine: an environmental risk assessment

María Luisa Lucero¹, Armin Peither², and Francisco Ledo¹

¹FAES Farma, S.A., Leioa-Bizkaia, Spain and ²Harlan Laboratories Ltd, Itingen, Switzerland

Abstract

Context: Bilastine is a new oral selective, non-sedating histamine H₁ antagonist for the symptomatic treatment of allergic rhinoconjunctivitis and urticaria. The European Medicines Agency requires an Environmental Risk Assessment (ERA) for all novel medicines for human use. **Objective:** To calculate the bilastine predicted environmental concentration in surface water (PEC_{sw}; phase I ERA), and to determine the effects of bilastine on aquatic systems (phase II [tier A]). **Materials and methods:** Bilastine PEC_{sw} was calculated using the maximum daily dosage (20 mg), assuming that all administered bilastine was released into the aquatic environment. A persistence, bioaccumulation and toxicity assessment was conducted using the log K_{ow} from the molecular structure. In phase II (tier A), a ready biodegradability test was performed, and bilastine's potential toxicity to various aquatic and sediment-dwelling micro-organisms was evaluated. **Results:** Bilastine PEC_{sw} was calculated as 0.1 µg L⁻¹, and the compound was not readily biodegradable. Bilastine had no significant effects on *Chironomus riparius* midges, or on the respiration rate of activated sludge. For green algae, the bilastine no observed effect concentration (NOEC) was 22 mg L⁻¹; bilastine had no effect on zebra fish development, or on the reproduction rate of daphnids. **Discussion:** Bilastine NOEC values against zebra fish, algae, daphnids, and aerobic organisms in activated sludge were at least 130 000-fold greater than the calculated PEC_{sw} value. **Conclusion:** No environmental concerns exist from bilastine use in patients with allergic rhinoconjunctivitis or urticaria.

Keywords

Antihistamine, biodegradability, ecotoxicology, toxicology

History

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Introduction

Bilastine is a novel, second-generation, histamine H₁-receptor antagonist marketed in several countries for the treatment of allergic rhinoconjunctivitis (seasonal and perennial) and urticaria at a maximum adult daily dosage of 20 mg day⁻¹ (Church, 2011; Medicines-Compendium-UK, 2012). In Europe, an Environmental Risk Assessment (ERA) is required by the European Medicines Agency (EMA) for all novel medicinal products for human use. The ERA includes evaluation of potential risks to the environment posed by novel drug compounds, and is a stepwise process with two phases. Phase I includes assessment of the predicted environmental concentration (PEC) of the test drug, with key assumptions that the sewage system is the principal route of test drug entry into surface water, and that the test drug is not biodegraded or retained in the sewage treatment plant; drug metabolism in the patient is not taken into account. In addition, with reference to the OSPAR Convention (OSPAR, 1992) and European guidelines (ECHA, 2014), drug substances with a log K_{ow} > 4.5 should be assessed, in a step-wise procedure, for persistence, bioaccumulation and toxicity (PBT). Phase II (tier A) is an environmental fate and effects analysis that incorporates

information about physicochemical and ecotoxicologic properties of the test drug in relation to environmental exposure (EMA, 2006).

The current study provides comprehensive data from an ERA of bilastine, with particular emphasis on calculation of PEC in line with a set of conservative, worst-case assumptions (phase I); and on fate and potential effects of bilastine in the sewage treatment plant and aquatic environment (phase II, tier A). Phase II of the ERA was designed to test the following for bilastine: biodegradability; potential effects on sediment-dwelling organisms; potential inhibitory effects on the respiration rate of aerobic waste water micro-organisms in activated sludge; effects on growth of the green algal species *Pseudokirchneriella subcapitata*; effects on early-life stage of the zebra fish *Danio rerio*; and effects on reproduction and survival of the aquatic invertebrate *Daphnia magna*. The bilastine ERA was approved by the EMA in September 2010. Partial results from the bilastine ERA were presented at the XII International Congress of Toxicology, Barcelona, July 2010 (Ledo et al., 2010).

Methods

All experimental procedures were performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice (GLP), adopted 18 May 2005 [RS 813.112.1]. This Ordinance is based on the OECD Principles of GLP, as

Address for correspondence: Francisco Ledo, FAES Farma, S.A., Autonomía no. 10, 48940 Leioa-Bizkaia, Spain. Tel: +34 94 481-8300. E-mail: fledo@faes.es

revised in 1997 and adopted 26 November 1997 by decision of the OECD Council [C(97) 186/Final]. These principles are compatible with GLP regulations specified by regulatory authorities throughout the European Community, the United States and Japan.

Calculation of predicted environmental concentration

In phase I of the ERA, exposure of the aquatic environment to bilastine was estimated by calculating the predicted environmental concentration (PEC) value. The following formula was used to estimate PEC in surface water (Equation 1) (EMEA, 2006):

$$PEC_{\text{SURFACE WATER}} = \frac{\text{dose}_{\text{ai}} \cdot F_{\text{pen}}}{\text{wastew}_{\text{inh}} \cdot \text{dilution}} \quad (1)$$

where dose_{ai} is the maximum daily dose of bilastine consumed per inhabitant; F_{pen} is the per cent market penetration; $\text{wastew}_{\text{inh}}$ is the amount (liters) of wastewater per inhabitant per day; and dilution is the dilution factor.

The calculation of $PEC_{\text{SURFACE WATER}}$ (PEC_{SW}) for bilastine was based on three very conservative, worst-case assumptions: (1) dose_{ai} was assumed to be the highest recommended daily dosage of bilastine (20 mg day^{-1}); (2) 100% of administered bilastine was assumed to be released into the aquatic environment (i.e. bilastine was considered to be non-significantly metabolized, to be eliminated mainly by fecal, and to a lesser extent renal, excretion and thereby to enter sewage treatment plants with subsequent release of effluents into the aquatic environment); and (3) an EMA default value for bilastine market penetration (F_{pen}) of 1% was employed. In addition, EMA default values were used for $\text{wastew}_{\text{inh}}$ (200 L per inhabitant per day) and dilution factor ($\times 10$) (EMEA, 2006).

Regarding action limits, if PEC_{SW} is $<0.01 \mu\text{g L}^{-1}$, and there are no other environmental concerns, it is assumed that the test drug is unlikely to pose an environmental risk after prescribed use in patients; conversely, if PEC_{SW} is $\geq 0.01 \mu\text{g L}^{-1}$, then a phase II environmental fate and effects analysis is needed (see subsequent methodologic sections) (EMEA, 2006).

Ready biodegradability test

Bilastine (in this and all subsequent tests detailed, provided by FAES Farma, S.A., Leioa-Bizkaia, Spain) was assessed for its ready biodegradability in a 28-day carbon dioxide evolution (modified Sturm) test conducted in line with internationally accepted guidelines and recommendations (OECD, 1992b).

The biodegradability test was performed with aerobic activated sludge from a wastewater treatment plant (ARA Ergolz II, Füllinsdorf, Switzerland) treating predominantly domestic wastewater. One day before test start, 2.4–3.0 L of untreated test medium was filled into 5 L amber glass flasks. An inoculum of activated sludge (90 mL) was added to each flask. The test media were aerated overnight with CO_2 -free air to purge the system of carbon dioxide. The next day, defined quantities of bilastine were added from a freshly prepared, concentrated stock solution (250.4 mg per liter). The reference item (sodium benzoate [Sigma-Aldrich International GmbH, St Gallen, Switzerland] 770 mg per 100 mL test water) was

tested simultaneously under the same conditions, and served as a procedural control. Two absorber flasks containing sodium hydroxide 0.05 M were connected in series to the exit air line of each test flask. All 5 L amber glass test flasks were incubated in a dark room at $21\text{--}23^\circ\text{C}$ for 28 days.

At the test start, inorganic and total carbon contents were measured in all test flasks. Subsequently, on each sampling day, 5 mL aliquots were withdrawn from the absorber flask nearest to the test flask for analysis of inorganic carbon (IC). Additional samples for the analysis of IC in all test vessels were taken from the second absorber flask at study end to correct for any carryover of CO_2 . Thus, absorber flasks for the bilastine tests were sampled on days 2, 5, 7, 9, 12, 14, 19, 23, and 27, and twice on day 28; and absorber flasks for the sodium benzoate (control) tests were sampled on days 2, 7, and 14, and twice on day 28. All samples were analyzed for IC using a Shimadzu TOC-5000A analyzer (Shimadzu Scientific Instruments, Columbia, MD).

The absolute amount of IC produced was calculated from the actual IC content in the absorber flasks plus the sum of IC removed in analytical samples. Per cent degradation was calculated from Equation (2):

$$\begin{aligned} \% \text{ degradation} &= \frac{\text{mg IC}_{\text{prod}} \text{ in test flask} - \text{mg IC}_{\text{prod}} \text{ in blank}}{\text{mg TOC}} \\ &\times 100\% \end{aligned} \quad (2)$$

where the conversion factor for CO_2 to carbon is 0.27; $\text{mg IC}_{\text{prod}}$ in test flask is the absolute amount of IC produced per test flask (i.e. mg carbon in the bilastine or sodium benzoate test flask); $\text{mg IC}_{\text{prod}}$ in blank is the absolute amount of IC produced (mg carbon) in flasks containing neither bilastine nor sodium benzoate; and mg total carbon (mg TOC) is 0.73 per mg of bilastine, or 0.58 per mg of sodium benzoate.

The ready biodegradability test was performed in compliance with GLP. Replicate tests were conducted for bilastine and control, and mean biodegradability was calculated.

Degradation and distribution in aquatic systems (river and pond)

Initial data from transformation studies in aquatic sediment systems indicate that bilastine does not persist in the water phase of the aquatic environment, but may remain in sediment for extended periods of time (FAES Farma SA, 2013) (Table 1).

Thus, as bilastine accounted for $>10\%$ of applied radioactivity in sediment extracts in previous tests conducted in line with OECD guidelines (OECD, 2002), further testing of the effects of bilastine on sediment-dwelling micro-organisms was needed. A test on sediment-dwelling larvae of the midge *Chironomus riparius* (Harlan Laboratories Ltd, Itingen, Switzerland) was therefore conducted according to OECD guidelines (OECD, 2004).

First-instar larvae of *C. riparius* were exposed to bilastine for 28 days to determine the impact on full maturation of the larvae to adult midges. That is, radiolabeled bilastine was applied to an artificial sediment, comprising sphagnum peat (5%), kaolin clay (20%), sand (75%), and trace amounts of

Table 1. Degradation and distribution of bilastine in aquatic systems (river and pond).

Bilastine	Water		Total System	
	River	Pond	River	Pond
DT ₅₀ (days)	6.1	6.7	107	62
DT ₇₅ (days)	12.3	13.4	214	124
DT ₉₀ (days)	20.4	22.2	356	206
r ²	0.976	0.960	0.745	0.659
Model used	SFO			

In aerobic aquatic systems, ¹⁴C-Bilastine rapidly dissipates from the water phase by adsorption to the sediment. Once in the sediment, its degradation proceeds at a slower rate, mainly via the formation of bound residues. DT_x: period in which x% of the initial dose is depleted or degraded; SFO: single first order kinetics.

calcium carbonate, and with a total organic carbon (TOC) content of 2.4% (based on dry weight), in static water-sediment systems. Five concentrations of bilastine were tested: nominal 6.3, 12.5, 25, 50, and 100 mg kg⁻¹ dry sediment; a control (water-sediment system without bilastine) was also tested. Analytically measured total radioactivity in the water-sediment systems ranged from 89–93% of applied radioactivity, thus confirming that the correct dosages of bilastine had been applied. Minor metabolites of bilastine were detected in the aqueous phase and sediment, but accounted for ≤0.3% and <3%, respectively, of the applied radioactivity.

The spiked sediments were kept at test conditions (i.e. water temperature 20 °C–21.7 °C; a 16-h light to 8-h dark photo-period) for 2 days, to reach equilibrium between sediment and aqueous phases, before introduction of the first-instar larvae. Tetra Min® fish food (Tetra-Werke, Melle, Germany) was added (23–47 mg per test vessel) at least three times per week until day 25, when all adult midges had emerged.

The endpoints of the study were emergence ratio (the sum of fully emerged midges divided by the number of inserted larvae), and development rate of the test larvae (the reciprocal of development time in days). Thus, the toxicity test was designed to determine the lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) of bilastine to *C. riparius*. The numbers of emerged adult midges, and their sex, were recorded daily from day 10 after larval insertion until day 28 (6 days after emergence of the last midges in the control). The numbers of visible pupae that failed to emerge were counted separately in each test vessel. Any other signs of intoxication of the larvae, pupae, and emerged midges were recorded. All test vessels were searched for deposited egg masses to prevent re-introduction of new larvae into the sediment.

All larval-sediment testing was performed in compliance with GLP. To obtain an approximate normal distribution, and to equalize variances, an arcsin-adjusted value was calculated for emergence ratio (ER_{arc}; Equation 3):

$$ER_{arc} = \arcsin(\sqrt{ER}) \quad (3)$$

where ER is the unadjusted emergence ratio.

Emergence ratio values in control vessels ranged from 75–85%, thus satisfying the OECD guideline validity criterion of

≥70%; in addition, adult midges in control vessels emerged between days 13 and 22, thereby fulfilling the OECD guideline validity criterion of emergence from day 12 to day 23 (OECD, 2004).

Mean emergence ratios and development rates for all bilastine test concentrations were evaluated by multivariate Dunnett's test after a one-way analysis of variance (ANOVA) to determine significant differences from control.

Respiration inhibition testing

The potential inhibitory effect of bilastine on the respiration rate of aerobic waste water micro-organisms in activated sludge was investigated in a 3-h respiration inhibition test conducted according to internationally accepted guidelines and recommendations (European-Communities, 1988; OECD, 2010).

A limit test was performed by adding bilastine at a nominal concentration of 100 mg L⁻¹ to the test solution comprising 1.0 g dry weight per liter of activated sludge from a wastewater treatment plant treating predominantly domestic waste. After 3 h incubation under aerobic conditions at 20 °C, the oxygen consumption rate of the micro-organisms was determined and compared to two untreated controls.

The dissolved oxygen concentration was measured with an oxygen electrode and meter (WTW TriOxmatic® 300 and WTW Oxi 539; Wissenschaftlich-Technische Werkstaetten WTW, Weilheim, Germany), and was continuously recorded. Oxygen consumption rate (mg O₂ L⁻¹ min⁻¹) was determined from the linear part of the respiration curve. The inhibitory effect of bilastine on respiration rate (oxygen consumption per minute) was expressed as per cent of the mean respiration rate in the two controls (Equation 4):

$$\% \text{ inhibition} = \left(1 - \left(\frac{2R_b}{R_{c1} + R_{c2}} \right) \right) \times 100\% \quad (4)$$

where R_b is respiration rate (mg O₂ L⁻¹ min⁻¹) at the tested bilastine concentration; and R_{c1} and R_{c2} are respiration rates in control vessels 1 and 2, respectively.

All aspects of the respiration inhibition test were performed in compliance with GLP.

Effects on algal growth

The influence of bilastine on growth of the green algal species *P. subcapitata* (strain number SAG 61.81; University of Göttingen, Göttingen, Germany) was investigated in a 72-h static test conducted according to internationally recognized guidelines and recommendations (European-Communities, 1992a; OECD, 2006)

Exponentially growing cultures of the unicellular algal species (inoculum 1.0 × 10⁴ cells mL⁻¹) were exposed to nominal bilastine concentrations of 4.6, 10, 22, 46, and 100 mg per liter of test medium; the bilastine tests were performed in parallel with a control. The test design included three replicates per bilastine test concentration, and six replicates of the control. Algal cell density in the test solutions was measured at 24, 48, and 72 h of exposure, whereas bilastine concentrations were determined in representative samples taken at 0 and 72 h. Bilastine concentrations in the analyzed test media ranged from 84 to 96% of nominal values at the

study start and end, thus demonstrating correct preparation of the test media, and bilastine stability in the media over the test period.

Algal biomass (algal cell density) was measured using an electronic particle counter (Coulter Counter® Model ZM; Beckman Coulter International S.A., Nyon, Switzerland); measurements were performed at least in duplicate. Inhibition of algal growth was determined from calculation of the specific growth rate (μ ; Equation 5) and yield (Y; Equation 6) over 72 h:

$$\mu_{0-72} = \frac{\ln X_{72} - \ln X_0}{72} \quad (5)$$

where μ_{0-72} is the average specific growth rate from time 0 to 72 h; X_0 is the biomass at time 0; and X_{72} is the biomass at time 72 h.

$$Y = X_{72} - X_0 \quad (6)$$

where Y is the yield; X_0 is the biomass (nominal value) at test start; and X_{72} is the biomass at test end.

Growth rate and yield were calculated for each test and control vessel. Mean values for growth rate and yield were then calculated for each bilastine test concentration and control. Mean values for inhibition of growth rate (I_r) and inhibition of yield (I_y) were calculated according to the following equations (Equations 7 and 8):

$$I_r = \frac{\mu_c - \mu_t}{\mu_c} \times 100\% \quad (7)$$

where I_r is % inhibition of average specific growth rate; μ_c is mean value for average specific growth rate in the control group; and μ_t is average specific growth rate for the bilastine test replicates.

$$I_y = \frac{Y_c - Y_t}{Y_c} \times 100\% \quad (8)$$

where I_y is % inhibition of yield; Y_c is mean value for yield in the control group; and Y_t is mean value for yield for the bilastine test replicates.

All aspects of the algal growth inhibition test were conducted in line with GLP. Statistically, for LOEC and NOEC values, average growth rate and yield for each bilastine test concentration were compared to control values using Dunnett's test.

Effects on zebra fish early-life stage

The effects of bilastine on zebra fish (*D. rerio*) were investigated in an early-life stage toxicity test conducted according to internationally recognized guidelines (OECD, 1992a)

Freshly fertilized eggs of the zebra fish (Hamilton-Buchanan 1822 strain; West Aquarium GmbH, Bad Lauterberg, Germany) were exposed under semi-static conditions to bilastine concentrations of 0.13, 0.41, 1.3, 4.1, and 13 mg L⁻¹. Tests were performed with 60 eggs per test (and control) group, divided into 4 replicates per group. Development and hatching of larvae, and survival and growth of juvenile fish, were investigated under defined conditions (water temperature 26.6°C, and a 16-h light to 8-h dark

photoperiod) over 35 days: i.e. 5 days post-fertilization, then 30 days post-hatching. Food for the larvae comprised live ciliate rotifers (Harlan Laboratories, Itingen, Switzerland) from day 6, dry food (Tetra-Werke, Melle, Germany) from day 8–17, and subsequently, hatched larvae of *Artemia salina* (Harlan Laboratories).

Bilastine solutions were freshly prepared on days 1, 6, 13, 21, and 27, and were applied using an automatic dosing system (Hamilton digital dispenser; Hamilton, Reno, NV) to 22 L flow-through aquaria, each containing four replicate test vessels. Bilastine concentrations in the application solutions and analyzed test media ranged from 91 to 104% of nominal values, thus confirming correct preparation and dosing, and satisfactory stability.

Recorded or calculated parameters comprised: hatching success (number of hatched larvae divided by number of inserted eggs, multiplied by 100%); development rate (i.e. reciprocal of hatching time; day⁻¹); survival rate at study end (number of surviving fish divided by number of hatched larvae, multiplied by 100%); and fish body length (mm), and wet and dry weights (mg) at study end. The mean wet weight of test fish was assessed for significant differences between the bilastine and control groups by the multivariate Williams test (Williams, 1971, 1972) after one-way ANOVA.

The early-life stage toxicity test was conducted in accordance with GLP, and the study was performed in line with Swiss Animal Protection Law.

Effects on reproduction of aquatic invertebrates

The effects of bilastine on survival and reproduction of females of the water flea species *D. magna* Straus (clone 5; University of Sheffield, Sheffield, UK) were investigated in a semi-static test over 3 weeks. The tests were conducted in line with GLP, and in line with internationally accepted guidelines and recommendations (European-Communities, 1992b; OECD, 2012)

The nominal bilastine concentrations tested were 1.0, 3.2, 10, 32, and 100 mg L⁻¹, and a control was tested in parallel. At the two highest test levels (32 and 100 mg L⁻¹), measured bilastine concentrations in the analyzed test media were 83–97% of nominal values, thereby confirming correct preparation of the test media and demonstrating sufficient bilastine stability during the test period.

Ten daphnids per treatment were tested, and each animal was kept individually in a 100 mL glass beaker containing 80 mL of test medium. Test vessels were covered with glass plates to reduce evaporative loss and prevent dust entry. Test media were renewed every 48–72 h in a temperature-controlled room, and water temperature in the test vessels was maintained at 20°C–21°C. A 16-h light to 8-h dark photoperiod was used, with light intensity of approximately 500–630 Lux. Test animals were fed daily (except day 3) with a food mixture containing a suspension of the green algal species *Scenedesmus subspicatus* (Harlan Laboratories, Itingen, Switzerland) and fish food (Tetra-min; Tetra-Werke, Melle, Germany). The total amount of food administered was equivalent to 0.2 mg total organic carbon per daphnid per day.

Test vessels were observed for the death of adult daphnids on days 0–2, and three times per week thereafter. The

reproduction rate was calculated as the total number of living offspring produced per parent female surviving until study end. Mean reproduction rates at the bilastine test concentrations were compared to control rates by multiple Williams' tests (Williams, 1971, 1972)

Results

Predicted environmental concentration

According to Equation (1), and with an assumed bilastine market penetration of 1% and dosage of 20 mg day⁻¹, together with default values of 200 L per inhabitant per day for the amount of wastewater, and 10 for dilution factor, bilastine PEC_{SW} was calculated to be 0.1 µg L⁻¹. This is greater than the action limit of 0.01 µg L⁻¹, such that a phase II environmental fate and effects analysis is needed for bilastine (see "Methods", and subsequent "Results" sections) (EMEA, 2006).

Ready biodegradability test

Bilastine was not readily biodegradable. After 28 days, mean bilastine degradation was negligible (2.7%), whereas that of the control substance (sodium benzoate) was almost complete (89.2%; Figure 1). Additional testing revealed that bilastine had no inhibitory effect on the activity of sludge microorganisms (FAES Farma SA, 2013).

Effects on sediment organisms

Mean arcsin-adjusted emergence ratios for male and female midges combined, and mean development rates for male and female midges considered separately, were not statistically or biologically significant for all bilastine concentrations versus control (Figures 2 and 3). Thus, bilastine had no adverse effects on emergence ratios and development rates at concentrations up to and including 100 mg kg⁻¹ dry sediment. The overall 28-day NOEC for bilastine was ≥100 mg kg⁻¹ dry sediment, and the overall 28-day LOEC

for bilastine was >100 mg kg⁻¹, although the latter value could not be quantified due to the absence of bilastine toxicity at the highest test concentration of 100 mg kg⁻¹ (Table 2).

Respiration inhibition testing

Bilastine, at the limit test concentration of 100 mg L⁻¹, had no significant inhibitory effect (−0.27% relative to controls) on the respiration rate of activated sludge after an incubation period of 3 h. Mean oxygen consumption rate was 1.467 mg O₂L⁻¹ min⁻¹ in the bilastine test, compared with a mean value of 1.463 mg O₂L⁻¹ min⁻¹ in the control test. Thus, 3-h NOEC for bilastine was at least 100 mg L⁻¹, and may have been even greater, although concentrations >100 mg L⁻¹ were not tested.

Algal growth inhibition

Bilastine had a significant inhibitory effect on average algal growth rate and yield, over the 72-h test period, at concentrations ≥46 mg L⁻¹ (one-sided Dunnett's test, α = 0.05). The 72-h LOEC for bilastine was therefore 46 mg L⁻¹. The 72-h NOEC for bilastine was 22 mg L⁻¹, since at concentrations ≤22 mg L⁻¹, the algal growth rate and yield after 72 h were not significantly lower than in the control group (Table 3).

Effects on zebra fish early-life stage

As shown in Table 4, bilastine had no effect on the development of zebra fish during the 35-day test period. For all recorded or calculated parameters, mean (±SD) values for bilastine were similar to those for control, and were without evidence of a concentration-effect relationship. The mean survival rate of juvenile fish at study end was actually slightly higher in all bilastine groups versus the control group (95–100% versus 92%). Further, no statistically significant differences were evident between

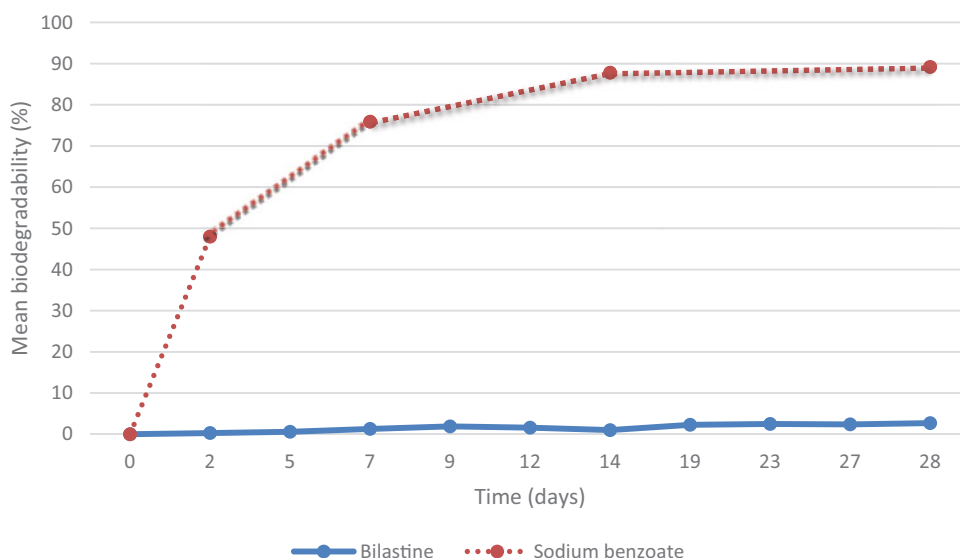


Figure 1. Mean biodegradability of bilastine in a modified Sturm test.

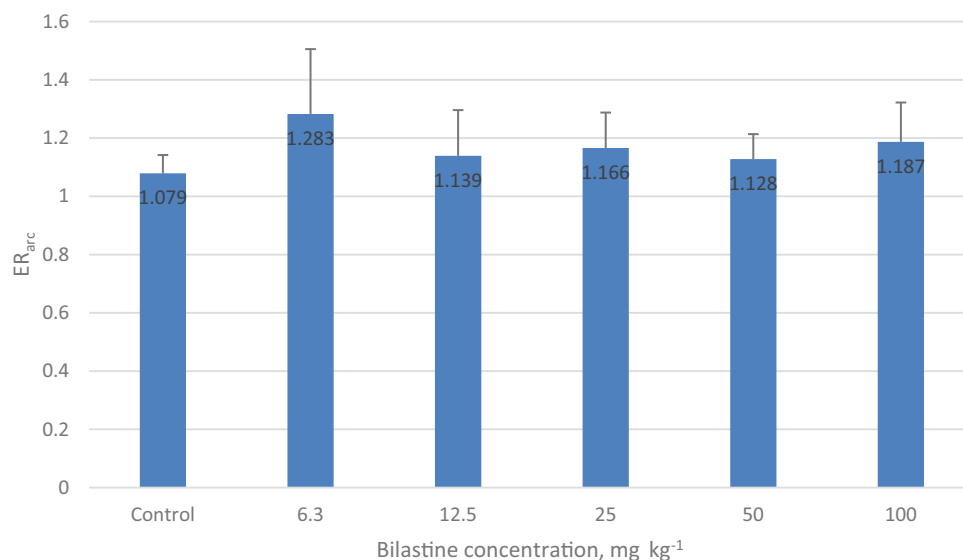


Figure 2. Effect of bilastine on arcsin-adjusted emergence ratio (ER_{arc}; mean ± SD) of the sediment-dwelling midge *Chironomus riparius*. Data for male and female midges combined.

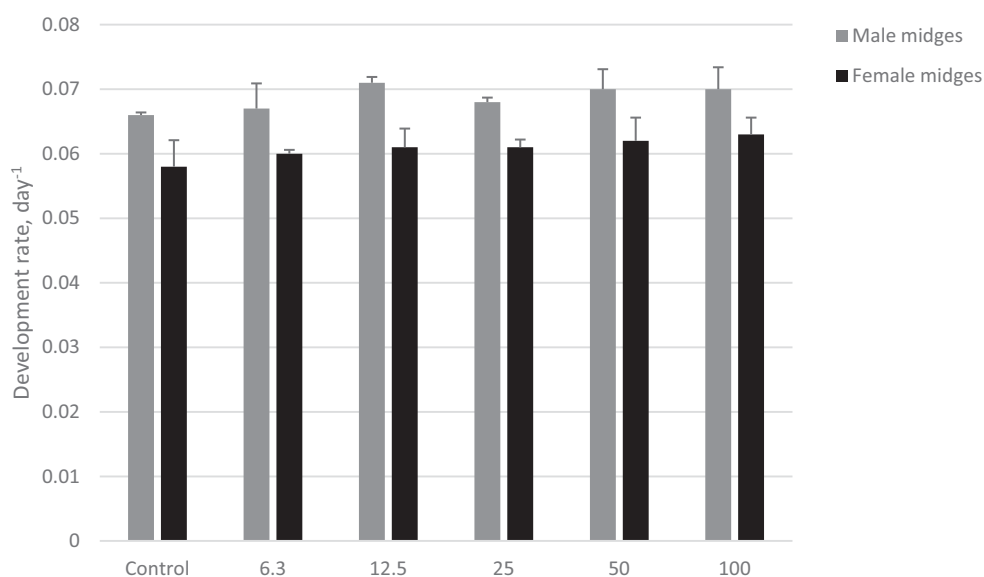


Figure 3. Effect of bilastine on development rates (mean ± SD) of male and female *Chironomus riparius* midges.

Table 2. Effect of bilastine on the emergence and development rate of sediment organisms.

Results after 28-days (mg/kg dry sediment)	Emergence rate (arcsin-transformed) of pooled sexes (mg/kg dry sediment)	Development rate (mg/kg dry sediment)	
		Males	Females
EC ₁₅	>100	>100	>100
EC ₅₀	>100	>100	>100
NOEC	100	100	100
LOEC	>100	>100	>100

bilastine and control regarding mean fish body length at study end (11.5–12.1 versus 12.2 mm). The overall NOEC for bilastine in this test was $\geq 13 \text{ mg L}^{-1}$; up to this level, no toxic effects were noted for bilastine on zebra fish eggs, larvae, or juvenile fish. The overall LOEC was $>13 \text{ mg L}^{-1}$.

Effects on reproduction of aquatic invertebrates

At bilastine concentrations $\leq 32 \text{ mg L}^{-1}$, and in the control group, daphnid survival at study end was $\geq 90\%$; however, in the bilastine 100 mg L^{-1} group, all daphnids were dead at day 7 (Figure 4).

Table 3. Effect of bilastine and control on mean growth rate (μ) and yield (Y) of green algae in a 72-h algal inhibition test.

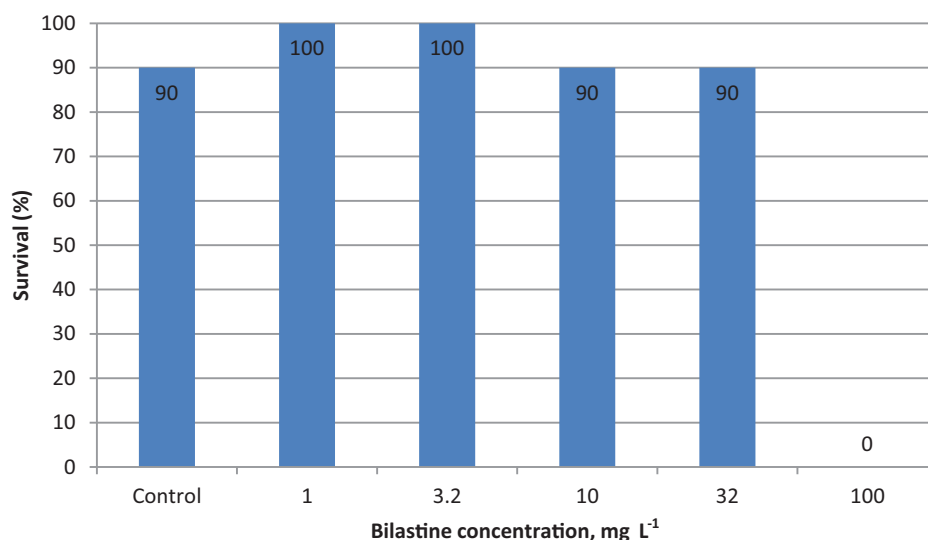
Nominal bilastine test concentration (mg L ⁻¹)	Mean growth rate (μ , day ⁻¹)	Inhibition of μ (I _{μ} , %)	Mean yield (Y, cells · mL ⁻¹ × 10 ⁴)	Inhibition of yield (I _Y , %)
Control	1.48	0.0	84.5	0.0
4.6	1.47	1.0	80.5	4.8
10	1.47	0.8	81.4	3.6
22	1.45	2.2	76.8	9.2
46	1.42*	4.1	70.1*	17.1
100	1.39*	5.9	64.7*	23.4

*Mean value significantly lower than in the control group (one-sided Dunnett's test, $\alpha = 0.05$).

Table 4. Bilastine had no major effects on zebra fish development in an early-life stage toxicity test.

	Control	Bilastine (nominal concentration, mg L ⁻¹)				
		0.13	0.41	1.3	4.1	13
Hatching success (%)	98.3 (2.9)	96.7 (5.8)	96.7 (3.3)	100 (0)	98.3 (2.9)	98.3 (2.9)
Development rate (day ⁻¹)	0.234 (0.0063)	0.232 (0.0095)	0.226 (0.0060)	0.230 (0.0072)	0.229 (0.0024)	0.231 (0.0082)
Survival at test end (%)	91.5 (2.8)	94.7 (3.1)	94.8 (5.9)	96.7 (3.3)	100 (0)	98.3 (2.9)
Body length at test end (mm)	12.2 (2.0)	11.5 (2.0)	11.9 (1.8)	11.8 (1.7)	12.1 (1.5)	11.6 (1.5)
Fish wet weight at test end (mg)	24.2 (6.8)	23.9 (7.3)	24.1 (6.4)	24.1 (5.9)	24.1 (4.7)	23.9 (5.9)
Fish dry weight at test end (mg)	5.0 (0.3)	5.0 (0.3)	5.0 (0.3)	5.1 (0.1)	5.1 (0.2)	5.0 (0.2)

Values shown are mean ($\pm$ SD).

Figure 4. Effect of bilastine on survival of *Daphnia magna* in a semi-static test over 21 days.

Mean ($\pm$ SD) reproduction rate of daphnids in the control test was 139.7 ± 11.5 living offspring per adult. Bilastine ≤ 32 mg L⁻¹ had no significant inhibitory effect on mean reproduction rate (Williams one-sided test, $\alpha = 0.05$), and no concentration-response relationship was noted (Figure 5). No reproduction occurred with bilastine 100 mg L⁻¹, since all daphnids in this test group were dead by day 7. The 21-day values for bilastine NOEC and LOEC were 32 mg L⁻¹ and 100 mg L⁻¹, respectively.

Discussion

Results from the entire ERA of bilastine confirm that the compound is not expected to have any adverse aquatic or

terrestrial (discussed later in this section) environmental effects when used for the treatment of patients with allergic rhinoconjunctivitis (seasonal or perennial) or urticaria.

Nevertheless, in phase I of the ERA (an estimation of environmental exposure), studies have extensively characterized the preclinical and pharmacokinetic profile of bilastine in various animal models and in humans (FAES Farma SA, 2013; Lucero et al., 2012). After oral administration of a 20 mg dose, bilastine was not significantly metabolized, and was largely eliminated unchanged in the urine (33%) and feces (67%). Excretion was completed within 7 days of administration, and after a radiolabeled dose, bilastine metabolites were reported to account for <4% (urine) and <1% (feces) of total radioactivity (FAES Farma SA, 2013).

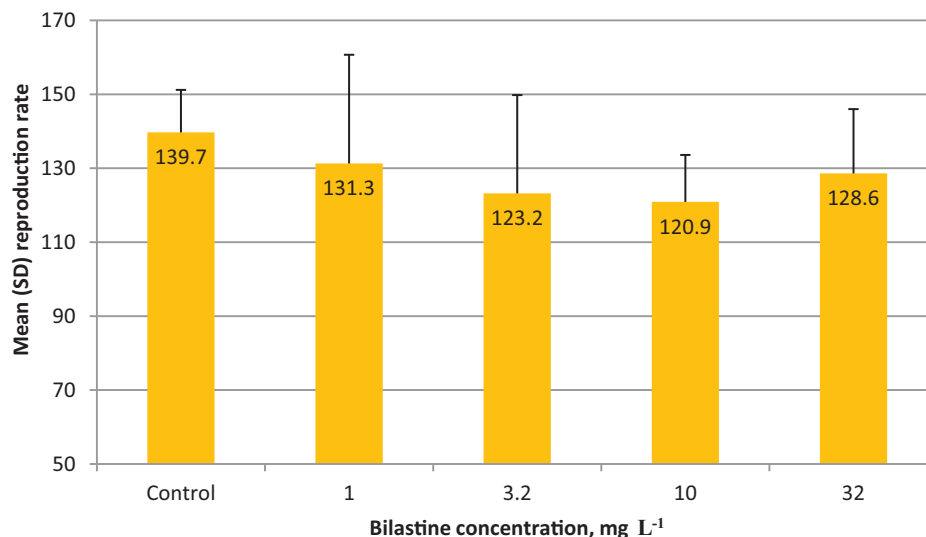


Figure 5. Effect of bilastine on mean reproduction rate of *Daphnia magna* in a semi-static test over 21 days.

Prior investigations have also shown that bilastine lacks interaction with cytochrome P450 metabolic pathways, an important characteristic for avoiding potential synergistic drug-drug interactions, indicating that bilastine is unlikely to affect the biotransformation of drugs administered concomitantly (Lucero et al., 2012). Taken together, these data demonstrate a lack of cumulative or synergistic effects with other drugs and chemicals, indicating that bilastine is a good choice for allergic patients receiving treatment for other concomitant disease, including those with renal or hepatic dysfunction.

Bilastine's pharmacokinetic profile, together with the calculated PEC_{SW} value of $0.1 \mu\text{g L}^{-1}$, outline that after excretion in the urine and feces of treated patients, bilastine may be transported to surface waters via sewage systems and sewage treatment plants. The PEC_{SW} value is 10-fold greater than the action limit stipulated in EMA guidelines (EMA, 2006), such that a phase II (tier A) environmental fate and effects analysis was needed. Conversely, a phase II (tier B) extended analysis was not necessary, since initial aspects of the tier A evaluation reveal that bilastine has a low likelihood for bioaccumulation: values for the *n*-octanol: water partition coefficient (K_{ow}) of bilastine at environmentally relevant pH values were considerably less than the EMA threshold of 1000 stipulated for tier B testing [calculations by ChemIDplus: Log K_{ow} is in the range of -0.45 (pH 0) to 1.65 (pH 14)]. In addition, in various tested soils and sludges, mean organic normalized adsorption coefficient values (K_{oc}) for bilastine were in the range $255\text{--}315 \text{ L kg}^{-1}$ (soils) or $65\text{--}96 \text{ L kg}^{-1}$ (sludges) (FAES Farma SA, 2013); these values were substantially below the EMA threshold of $10\,000 \text{ L kg}^{-1}$, thus suggesting that no strong binding of bilastine to sewage is expected, and that tier B testing in the terrestrial environment and calculation of a PEC in sludge is not necessary for bilastine (EMA, 2006; FAES Farma SA, 2013). In other words, the potential for exposure of the terrestrial environment to bilastine from spreading of sludge on agricultural land for manuring is regarded as negligible.

All default values (e.g. dilution, market share) used in phase I of the ERA to assess exposure of the aquatic

environment to bilastine were based on EMA (EU Technical Guidance Document) guidance at the time of the analyses (EMA, 2006). As the aim of the study was to provide a rational basis for an evaluation of the potential bioaccumulation of bilastine, an assessment of, for example, different dilutions (e.g. highly effluent-dominated streams with a dilution <10 , or even 0 in some arid regions) was not undertaken in the current analyses.

The current study focused on several key aspects of the phase II (tier A) ERA for bilastine. The novel antihistamine was not readily biodegradable, and although earlier studies reported that the compound may remain in sediment for prolonged periods, in the current trial, bilastine had no toxic effects on the development of sediment-dwelling larvae of the midge *C. riparius* in water-sediment systems ($NOEC \geq 100 \text{ mg kg}^{-1}$ dry sediment). Moreover, bilastine was relatively non-toxic to various micro- and other organisms, with $NOEC$ values ranging from $\geq 13 \text{ mg L}^{-1}$ (zebra fish), to 22 mg L^{-1} (algae), to 32 mg L^{-1} (daphnids), and $\geq 100 \text{ mg L}^{-1}$ (aerobic wastewater micro-organisms in activated sludge). Of particular importance, these $NOEC$ values are many orders of magnitude (i.e. at least 130 000-fold) greater than the calculated PEC_{SW} value for bilastine of 0.0001 mg L^{-1} . EMA guidelines (EMA, 2006) stipulate that a phase II (tier B) ERA is needed when $PEC_{SW}:NOEC_{\text{micro-organism}}$ is greater than 0.1; in the current trial, however, the greatest $PEC_{SW}:NOEC_{\text{micro-organism}}$ ratio for bilastine was at least 10^4 times less than the EMA stipulation. It is important to note that assessment factors are used to calculate the PEC/PNEC ratio prior to assessing the risk for the respective species group (EMA, 2006).

Given that bilastine represents a new chemical entity, there is currently no literature regarding ecotoxicological studies of substances with similar chemical structures. Nevertheless, the aquatic toxicology of diphenhydramine, a first-generation antihistamine, has been reported recently (Berninger et al., 2011); data showed that an aquatic plant model was insensitive to diphenhydramine, whereas significant acute and subchronic effects of diphenhydramine were observed in a model fish (*Pimephales promelas*) and the invertebrate

D. magna, possibly related to acetylcholine activity, an alternative mechanism of action (MoA) (Berninger et al., 2011).

With this in mind, it is noteworthy that there is an increasing body of evidence within the pharmaceuticals and personal care products community to consider MoA-specific effects on non-target organisms using the adverse outcome pathway (AOP) concept (a sequence of key events from an initial molecular-level event and an ensuing cascade of steps to an adverse outcome with population-level significance) and the “read-across” hypothesis (Ankley et al., 2010; Berninger & Brooks, 2010; Brausch et al., 2012; Kramer et al., 2011; Watanabe et al., 2011). However, it is important to note that AOP protocols have not been standardized or recognized internationally; whilst our current work focuses on gross biological endpoints, as mandated by regulatory authorities, future MoA-based research efforts with bilastine using the AOP framework would be valuable.

Conclusion

Based on all data obtained, exposure of the terrestrial and aquatic environments to bilastine is expected to be insignificant.

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