COMBINED ENVIRONMENTAL RISK ASSESSMENT FOR THE ANTIVIRAL PHARMACEUTICALS GANCICLOVIR AND VALGANCICLOVIR IN EUROPE

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Abstract: Potential environmental risks of the old antiviral pharmaceuticals ganciclovir (GCV) and valganciclovir (VGCV) were reassessed based on new environmental fate and chronic ecotoxicity tests and on actual use data for Europe. Ganciclovir is hydrolyzed to GCV by intestinal and hepatic esterases, and hence the new environmental tests only refer to GCV. A sorption study showed that GCV will not sorb significantly, excluding the soil as a relevant environmental compartment. Despite earlier data suggesting nondegradability, a new water/sediment fate test showed GCV to be primarily and ultimately degraded and to be nonpersistent. The chronic ecotoxicity tests with algae and daphnids resulted in no inhibition at the highest tested concentrations, whereas a fish partial life cycle test, selected in view of mammalian mutagenicity and reprotoxicity data, showed effects on growth of the young fish, but not on gametogenesis, fertilization, embryogenesis, or teratogenicity. Predicted environmental concentrations were derived based on actual per capita use data for European countries for 2004 to 2014, and the highest was selected for the risk assessment. A comparison of predicted environmental concentrations with predicted no-effect concentrations shows no significant risk for wastewater treatment, surface waters, groundwater, or sediment. In addition, potential risks to (semi)aquatic top predators or to human consumers of water and fish are exceedingly low.

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INTRODUCTION

The presence and potential risks of pharmaceuticals in the environment are of great scientific and regulatory interest, particularly the older, so-called legacy active pharmaceutical ingredients (APIs). These are particularly of interest because it is mainly the legacy APIs that are detected in environmental matrices, whereas environmental fate and toxicity information in the public domain is often lacking. Two such APIs are the closely related ganciclovir (GCV) and valganciclovir (VGCV), which have been used for the treatment and prevention of cytomegalovirus and other herpesvirus infections since the 1990s [1].

For both GCV and VGCV, only an older Roche internal environmental risk assessment (ERA) [2] is available, without chronic effects or complete fate data. However, the API GCV was found to be mutagenic in a mouse micronucleus test and reprotoxic in mammals (teratogenic in rabbits) [3,4]. Based on this information, GCV was classified as carcinogenic, mutagenic, or reprotoxic (CMR) in the European Union. Therefore, the old ERA was updated with new environmental fate and effects tests, the latter specifically including reprotoxicity endpoints in aquatic organisms, and was based on documented sales figures for Europe for GCV and VGCV.

MATERIALS AND METHODS

Substances and basic properties

Ganciclovir is 9-(1,3-dihydroxy-2-propoxymethyl)guanine (Figure 1), a synthetic guanine derivative with the Chemical Abstracts Services (CAS) number 82410-32-0, a molecular mass of 255.23 g/mol, the empirical formula C₉H₁₃N₅O₄, and a melting/decomposition temperature range of 242 °C to 255 °C [3]. It has 2 dissociation constants, an acid pKa of 9.57, and a base pKa of 2.2 [3], meaning that GCV is mainly unionized in the pH range between 4 and 8. This explains the uniformly low n-octanol/water distribution coefficients (log Dₕᵢw) of −1.96, −1.94, and −2.00 at pH 5, 7, and 9, respectively [3], where the average measured log Dₕᵢw of −1.95 at pH 5 and 7 corresponds to the un-ionized partition coefficient (log Kₒᵢw) of GCV. The aqueous solubility of GCV is given as 2760 mg/L at pH 7 [3]. Roche Cymeneve’ and Cytovene’ is GCV sodium (CAS no. 84245-13–6) with a mass of 277.21 g/mol [5].

Valganciclovir (CAS no. 175865–60–8) is the L-valine ester of GCV (Figure 1) with a molecular mass of 354.36 g/mol and the empirical formula C₁₁H₂₂N₅O₅ [6]. Roche Valcyte’ is VGCV hydrochloride (CAS no. 175865–59–5) with a mass of 390.88 g/mol [6]. It is not stable in aqueous, particularly neutral and alkaline, solutions but hydrolyzes to GCV and L-valine [6].

Ganciclovir and later VGCV were originally developed in the 1990s by Syntex (Palo Alto, CA, USA), which is now part of the Roche Group. Ganciclovir, the L-valine ester and prodrug of GCV, was developed because it shows much improved oral bioavailability compared with GCV. After oral ingestion, VGCV is rapidly hydrolyzed to GCV and L-valine by enteric and hepatic esterases; there is essentially no further metabolism, and GCV is excreted by the urinary pathway (Roche internal data [4]). Ludzack and Ettinger found that L-valine was rapidly degraded in an activated sludge system.
with 99% removal in 1 to 5 d [7] and, as a natural amino acid, it is not expected to cause significant toxicity. Thus, the present ERA is based on environmental data for GCV.

Environmental fate tests

New environmental fate and toxicity tests were performed at Smithers Viscient contract laboratories (Harrogate, UK) under Good Laboratory Practice (GLP) quality assurance and following Organisation for Economic Co-operation and Development (OECD) test guidelines [8]. Available older experimental data suggest some potential for biodegradation for GCV [3], but without matching the OECD criteria for ready biodegradability. Therefore, a sediment-water environmental fate test according to OECD guideline 308 using 2 natural sediment-water systems (Swiss Lake, Chatsworth, Derbyshire, and Calwich Abbey Lake, Calwich, Staffordshire, UK) was performed with GCV [14C]-radiolabeled on the pyrazole ring under GLP [9]. Detailed test methods for all experiments and for the analytical methods are given in the Supplemental Data. Based on 14C total mass balances and recoveries in the water, sediment extractables, nonextractable residues, and evolved 14CO2 over time as well as liquid chromatography–tandem mass spectrometry (LC–MS/MS) analytics of the water and sediment extracts, the distribution, degradation, nonextractable residues formation, and overall environmental fate of [14C]-GCV were characterized, and a description of a degradation pathway was attempted. Degradation rates for GCV were determined according to the FOCUS model [10] recommendations using CAKE (Tessella) Ver 2.0 software, which calculated the degradation rates and associated parameters. Dissipation from water, dissipation from sediment, and degradation in the total system were modeled using single first-order, first-order multi-compartment, and double first-order in parallel kinetics, based on measured GCV concentrations.

To predict environmental phase transfer through adsorption, an adsorption/desorption test was performed following OECD guideline 106 with [14C]-GCV as described above under GLP [11]. The substrates were 2 soils, 2 sediments, and 2 activated sludges, all sampled from England and characterized as to soil or sediment type, organic carbon content, and pH. An adsorption phase of 24 h was followed by a single desorption phase of 24 h. Based on the data, adsorption partition coefficients (Kd), organic carbon–normalized adsorption coefficients (KOC), the corresponding Freundlich adsorption coefficients (KF and KFOC), and the respective desorption coefficients were determined for each soil, sludge, and sediment as per the OECD guideline.

Actual use of GCV and VGCV and predicted environmental concentrations

We acquired IMS Health marketing data in kg per annum for Europe for the period 2004 to 2014 [12] to derive a use-based predicted environmental concentration (PEC) for European surface freshwaters. Because VGCV is metabolized to and excreted as GCV quantitatively, both products are covered for the European countries.

Although some (little) GCV is phosphorylated and subsequently integrated into herpesvirus DNA, thereby blocking viral replication, most is excreted without being metabolized [4]. Therefore, as a worst-case assumption, 100% excretion of GCV (from GCV and VGCV uptake) was assumed. Thus, the administered amounts of GCV plus the stoichiometric fractions of GCV excreted from VGCV administration for the European countries Austria, Belgium, Bulgaria, Croatia, Czechia, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Luxembourg, The Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, and the United Kingdom in the period 2004 to 2014 [12] were added by country and year, and then divided by the population (Eurostat [13]) for the respective country and year, by 365 d/annum and by the European Union defaults of 200 L of sewage per inhabitant and day as well as a default surface water dilution factor of 10 [14], to calculate a use-based surface water PEC for total GCV. Note that this PEC does not incorporate any removal during sewage treatment. For the ERA, the country with the highest per capita use was determined, and the European maximum-use surface water PEC was calculated for every year. The highest of these maximum-use surface water PECs over the whole decade was used for the ERA. The wastewater treatment PEC was back-calculated from the surface water PEC by multiplication with the dilution factor of 10. The groundwater PEC was derived following the European Medicines Agency ERA guideline as the surface water PEC divided by 4 [14].

Lastly, a sediment PEC was calculated using a nested formula (equation R.16–41) in the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) technical guidance document (part B, chapter 16 [15]). In view of many default values in this equation, this PEC ultimately depends on the organic-carbon/water distribution coefficient KOC and on the surface water PEC. The sediment PEC for the ERA was derived from the maximum KOC of all substrates in the adsorption/desorption test [11] as a worst-case assumption.

Environmental toxicity tests

The toxic potential of GCV for environmentally relevant organism groups was tested with new OECD tests for activated sludge respiration inhibition including nitrification inhibition (OECD 209); with an algal growth inhibition test (OECD 201); with a chronic daphnid reproduction test (OECD 211); and, in view of the mutagenicity and the reprotoxicity in mammals, with a fish partial life cycle test consisting of a fish short-term reproduction test (OECD 229) directly followed by a fish early life stage test (OECD 210). In addition, a chironomid development and emergence test (OECD 218) was performed to assess sediment toxicity. All tests were performed with analytical determination of the test concentrations (except for the OECD 209 test), and detailed test methods are given in the Supplemental Data.
An activated sludge respiration inhibition test including nitrification inhibition according to OECD 209 [16] was performed under GLP with GCV of a purity of 99.6%, with activated sludge from Burley Menston (UK) Sewage Treatment Works receiving predominantly domestic wastewater over 3 h to determine total respiration. The nitrification inhibitor N-allylthiourea was added to appropriate test and reference vessels to measure the carbon respiration only. In addition, 6 blank controls were prepared to allow measurement during the test series. The percentage inhibition of total, heterotrophic, and nitrification-related oxygen consumption was determined based on the measurement data following OECD guideline 209.

An algal growth inhibition study according to OECD guideline 201 over 72 h was performed under GLP with the green alga *Pseudokirchneriella subcapitata* (strain 278/4, Culture Collection of Algae and Protozoa, Ohan, UK) over 72 h [17] with GCV of a purity of 99.6%. Based on a pretest, the definitive test was conducted as a limit test at a single nominal concentration of 100 mg/L in 6 test vessels. A control group of 6 test vessels was also included. Each test vessel was inoculated with 10⁴ algal cells/mL and incubated at 21 to 24°C in an algal cabinet under continuous 4440 to 8880 lux light intensity for 72 h, with cell counts (Z2 Coulter Counter; Beckman Coulter) at 24-h intervals. The LC–MS/MS analysis of the test media samples was conducted at 0 h and 72 h. Depending on the cell counts over time, effects were determined or calculated as a no-observed-effect concentration (NOEC) or as an EC₅₀ effect concentration, where x is 10 or 50, relating to yield or growth rate according to OECD guideline 201.

A daphnid reproduction test using *Daphnia magna* according to OECD guideline 211 over 21 d was performed with GCV of a purity of 99.6% [18], using a semistatic design with renewal of the test media 3 times a week. The definitive test was conducted at nominal concentrations of 0 (control group), 0.032, 0.10, 0.32, 1.0, and 3.2 mg GCV/L; the applied test concentrations are based on a pretest. Ten replicate test vessels were prepared for the control and each test concentration. At the end of each exposure period, water quality and temperature measurements were determined using pooled replicate samples of test media at each of the nominal concentrations. The test was conducted with a 16:8-h light:dark cycle with an approximate 30-min dawn/dusk transition period. Test concentrations were analytically determined by LC–MS/MS. At the start of the test, a single juvenile *D. magna* (<24 h old) was randomly allocated to each test vessel. The daphnids were fed daily with a suspension of *Chlorella vulgaris*. The adult daphnids were observed daily for immobility, the presence or absence of eggs developing in the brood pouch (gravid or nongravid), and mortality throughout the duration of the test. In addition, the number of juveniles present (alive or dead) was recorded daily. On medium renewal occasions, parental animals were transferred into freshly prepared test media. At the end of the test, the carapace lengths of all surviving parental *D. magna* were measured. In a statistical analysis to determine the NOEC and lowest-observed-effect concentration (LOEC), the total number of juveniles produced by surviving adults by day 21, the final adult lengths, and the intrinsic rate of population increase were analyzed using the Bonferroni-adjusted t test.

Ganciclovir is CMR in mammals and therefore potentially also reprotoxic or mutagenic in fish. Thus a partial fish life cycle test was performed using *Pimephales promelas* (fathead minnow) by combining a fish short-term reproduction test according to OECD guideline 229 with a fish early life stage test (OECD 210) with the same test material as above under GLP [19]. The reproduction test phase exposes actively spawning adults (F0 generation) to 3 different GCV concentrations; the F0 generation was assessed for reproductive success over a 21-d period. At the end of the 21-d period, the adult fish were assessed for secondary sex characteristics and vitellogenin. In addition, eggs produced by the F0 generation were incubated at the same GCV concentration of the respective F0 generation adults to assess the effects on the second (F1) generation. The F1 generation was exposed for 28 d posthatch (total F1 exposure duration 35 d) and assessed for hatching success, malformations, posthatch survival, and growth. This testing strategy was selected based on the reasoning that potential mutagenicity of GCV would result in disruption of gametogenesis in the F0 adults and thus in reduced spawning and fertilization success in the short-term reproduction test phase, while the early life stage test phase would uncover potential mutagenicity or developmental adverse effects of GCV over early development, including cell divisions, germ layer, and organ formation, up to hatching and resorption of the yolk sac to free-swimming young fish. Based on the results of a range-finding test, nominal concentrations of 0.010, 0.10, and 1.0 mg/L GCV were used for the definitive test. Four replicate vessels were used for each test concentration and for the control group. The test was conducted under continuous flow conditions. Test vessels were glass aquaria, except for the egg exposure, for which large-diameter glass tubes with an inert fine mesh at the bottom allowing water circulation were used as hatching vessels. After hatching, the young fish were moved to glass aquaria. Chemical analysis by LC–MS/MS of the GCV concentrations in the test and control vessels was conducted weekly throughout the duration of the test.

**OECD 229 short-term reproduction test phase.** In a 3-wk pre-exposure period, adult fathead minnows from the laboratory’s own breeding stock were kept under supervision in vessels and conditions like those used later in the actual test, but without exposure to GCV, to ensure that the fish were spawning successfully. Following this pre-exposure period, actively spawning adults (2 males and 4 females each per test vessel) were introduced into the test aquaria that contained one-half of an inert polyethylene tube as a spawning substrate. Any mortality was recorded, and the sex of the dead fish was determined by macroscopic examination of the gonads. Any abnormal behavior compared with controls (e.g., territorial aggressiveness, changes in body color, colorations, or shape) was also recorded. The spawning substrates were examined daily for the presence of eggs. The presence or absence of eggs was recorded, the number of eggs was counted, the eggs were removed, and the spawning substrate was returned to the test vessel. After 21 d, all F0 generation fish were killed in accordance with applicable animal protection legislation in the United Kingdom. The phenotypic sex of each fish was identified based on secondary sex characteristics (e.g., body shape and size, urogenital papillae, fat pad, nuptial tubercles). Blood was collected from each fish and centrifuged, and the plasma was submitted to vitellogenin analysis by enzyme-linked immunosorbent assay.

**OECD 210 early life stage test phase.** From the F0 generation, 4 spawns per concentration were incubated to assess the F1 larval/juvenile fish development. Nominally 20 viable eggs were added to each egg hatching vessel. Nonviable or necrotic eggs were removed, avoiding disturbance of adjacent viable eggs. The posthatch phase started once all of the viable eggs were considered to have hatched. Following hatching, the F1 generation fish were fed live brine shrimp (*Artemia sp.*) nauplii 1 to 3 times a day. At the start of the posthatch phase, an initial estimate of hatching success was made, and on day 28 posthatch, hatching success was confirmed...
by definitive counts of all surviving fish. On day 28, the total numbers of surviving larvae were counted, and individual total fish lengths and wet weights of all remaining fish were determined. The percentage of posthatch survival was determined by expressing the number of surviving larvae on day 28 posthatch as a percentage of the hatched larvae on day 0.

Reproduction parameters for the F0 generation (number of eggs and number of spawns per surviving female) as well as lengths and weights of both male and female adults at termination were analyzed using Dunnett’s multiple comparison test. The vitellogenin analysis results for the male and female fish were analyzed separately; for the female fish, the results were analyzed using Dunnett’s multiple comparison test, and for the male fish, the results were analyzed using the Jonckheere–Terpstra stepdown test. The F1 generation hatching success was analyzed using Steel’s many–one rank sum test. The 28-d posthatch survival and final lengths and weights were analyzed using Dunnett’s multiple comparison test.

An OECD guideline 218 chironomid development and emergence test using spiked sediment was performed under GLP with the midge Chironomus riparius over 28 d in artificial water/sediment systems [20] with both regular GCV test material and radiolabeled GCV as in the OECD guideline 308 test. Based on the results of a range-finding test, a definitive test was conducted at nominal concentrations of 0.32, 1.0, 3.2, 10, 32, and 100 mg GCV/kg sediment dry weight; a control group was also included. Four replicates were prepared for the control and each test concentration. The test was conducted in glass beakers (600 mL, with clear plastic lids) containing a layer of sediment (100 g) and water (400 mL) to give a sediment to water depth ratio of 1:4. After approximately 24 h of acclimation of the test vessels, 20 first-instar stage chironomid larvae from an in-house breeding population were added below the surface of the test media in each vessel, and the vessels were covered with a fine mesh. Additional test vessels were prepared in the same manner as the test vessels, without the addition of larvae, for use as so-called destructive samples for the analysis of sediment on day –1 (sediment fortification) for the control and for all test concentrations as well as sediment, overlying water, and porewater on day 0 (larval addition) for the control and each test concentrations. The test vessels were maintained in a temperature-controlled laboratory at 18 to 22 °C. Day length was controlled to give a 16:8-h light:dark photoperiod. In each test vessel the pH, temperature, and oxygen concentration were recorded. Samples of sediment, overlying water, and porewater from each test concentration were analyzed for total radioactive residues by liquid scintillation counting following combustion of the sediment samples. In addition, samples from the 100 mg/kg sediment vessels on days 0 and 28 were extracted and analyzed by LC–MS/MS to confirm the presence of parent test substance. Chironomids were exposed to the test or control conditions for a 28-d period, without renewal of the test medium. The larvae were fed daily, and the test vessels were observed daily for signs of larvae and tube formation. From day 14, when the first midges emerged, adults were sexed and removed from the vessels on a daily basis until the end of the test. The 28-d percentages for emergence success, development rate, and sex ratio were then calculated for each treatment. Statistical analysis was performed, and day 28 cumulative male and female emergence totals compared with controls were analyzed using a Chi-squared test; the emergence ratio values were first transformed and then analyzed using Dunnett’s multiple comparison test; the mean development rate per vessel was determined following OECD guideline 218, and the calculated development rates were then statistically analyzed using Dunnett’s multiple comparison test.

**Predicted-no-effect concentration derivations**

Predicted-no-effect concentrations (PNECs) were derived using assessment factors from the European Medicines Agency ERA guidance [14]. For deriving the sewage works PNEC, the NOEC from the OECD guideline 209 activated sludge respiration inhibition test was used by dividing by an assessment factor of 10 [14]. For the surface (fresh)water PNEC, the lowest NOEC from the chronic OECD guideline 201 algal growth test, the OECD guideline 211 daphnia reproduction assay, and the fish partial life cycle test (OECD 229 plus OECD 210) was divided by an assessment factor of 10 [14].

However, as recognized by the European Commission [21], this aquatic PNEC is not necessarily protective for (semi) aquatic mammalian or avian top predators. Therefore, a maximum tolerable daily intake (MTDI) for mammalian top predators such as otters was calculated following the algorithm of Murray-Smith and colleagues from AstraZeneca [22]. Briefly, acute and long-term mammalian toxicity results are divided by an appropriate assessment factor, depending on the nature and duration of the tests, to derive an MTDI. Then, for the case of an otter as a top predator with a default body mass of 10 kg, an intake of 1 kg fish and 0.79 L of water per day is assumed; the concentration of the substance in fish is based on the surface water PEC and the bioaccumulation factor. The combined daily intake of an otter from fish and water is then compared with the MTDI [22].

The groundwater PNEC was derived from the chronic daphnia (OECD 211) NOEC by dividing by an assessment factor of 10 [14]. Lastly, for the sediment PNEC, the chronic OECD guideline 218 chironomid development and emergence NOEC, referring to sediment dry weight, was first normalized to a standard sediment organic carbon content of 10% [23], which was then divided by an assessment factor of 100 in view of 1 chronic sediment NOEC being available [14].

Lastly, a potential surface water risk from indirect exposure of human consumers from drinking water, approximately 40% of which is produced from surface water in Europe [24], and from fish, which may bioaccumulate substances, is derived following Murray-Smith et al. [22]. Briefly, assuming as a worst case that GCV is not removed during drinking water production, the surface water PEC is taken as the drinking water PEC, and a default volume of 2 L drinking water consumption per day is assumed. For fish consumption, bioaccumulation from surface water into fish is considered (PEC × BCFfish [with BCF being the bioconcentration factor]), and a default amount of fish of 0.115 kg/d is assumed [22]. The combined resulting daily uptake of GCV from drinking water and fish consumption is compared with the acceptable daily oral intake for GCV [4].

**Environmental risk assessment**

Potential risk to the environmental compartments sewage treatment, receiving waters, groundwater, and sediment was assessed by deriving the respective PEC/PNEC risk characterization ratios, following the REACH and European Medicines Agency ERA procedures [14,15]. Other environmental compartments were included or excluded from the assessment as per the European Medicines Agency ERA [14] thresholds or criteria. Potential risks to top predators or human consumers of drinking water and fish were assessed by dividing the surface water PEC by the respective acceptable daily intake for GCV for top predators and humans.
Environmental persistence, bioaccumulation, and toxicity assessment

In view of the CMR properties of GCV as a result of mutagenicity and mammalian reprotoxicity, a persistence, bioaccumulation, and toxicity (PBT) assessment was performed, with special emphasis on potential reprotoxic properties. Persistence of GCV was assessed using the results of the OECD guideline 308 water/sediment transformation study. This laboratory study was performed at 20 °C, while the REACH technical guidance document thresholds for persistence of 40 d for freshwater and 120 d for freshwater sediment and for high persistence of 60 d and 180 d, respectively, refer to a default European environmental temperature of 12 °C [25]. The European Chemicals Agency (ECHA) Guidance on Information Requirements and Chemical Safety Assessment (Chapter R.7b, page 191) [26] gives an equation to approximate half-life (50% dissipation time [DT50]) rates for other temperatures

\[ \text{DT50} (T °C) = \text{DT50} (20 °C) \times e^{(0.08[T−20])} \]

where \( T \) is the tested and \( X \) is the target temperature. Thus, for an extrapolation from 20 °C to 12 °C, the equation is

\[ \text{DT50} (12 °C) = \text{DT50} (20 °C) \times 1.8965 \]

The potential for bioaccumulation of GCV was assessed using the European Medicines Agency ERA guideline screening information criterion of log \( K_{ow} > 3 \) [14]. Lastly, the potential for high ecotoxicity of GCV was assessed based on the results of the new surface water chronic ecotoxicity tests, particularly the fish partial life cycle test, and the REACH technical guidance document criterion for toxicity of an aquatic NOEC < 10 μg/L [25]. According to the REACH technical guidance document, a substance must conform to the criteria for persistence and bioaccumulation and toxicity to be classified as a PBT substance [25].

RESULTS

Environmental fate

In the water/sediment study [9] in Calwych Abbey and Swiss Lake systems (Figure 2), the overall mass balance was good, with all \(^{14}C\) values > 90% of dosed throughout, thus fulfilling the validity criteria for the test. At 20 °C, the \(^{14}C\) signal dissipated rapidly from the water phase, with DT50s of approximately 14 d for both systems and day 100 final \(^{14}C\) values of 4.6% for Calwych Abbey and 0.8% for Swiss Lake. Sediment-extractable \(^{14}C\) increased up to days 14 to 30 to 26.5% in Calwych Abbey, and 18.3% in Swiss Lake, and then declined slowly to 14.6% (Calwych Abbey) and 3.5% (Swiss Lake) by day 100. The sediment non-extractable residues \(^{14}C\) signal increased more slowly, to peak on day 62 with 40.1% (Calwych Abbey) and 29.6% (Swiss Lake), with final values decreasing to 37.8% (Calwych Abbey) and 22.6% (Swiss Lake); note that only so-called soft acetonitrile extraction was used. Evolved \(^{14}CO_2\) in the KOH traps increased continuously from day 7 to reach 37.3% (Calwych Abbey), and 69.5% (Swiss Lake) by day 100; no organic volatiles were recorded [9]. The DT50 and DT90 values for GCV at 20 °C derived from the test for the different compartments are given in Table 1, as are the DT50 values extrapolated for 12 °C and the European Union persistence criteria for water and sediment.

By LC–MS/MS analysis, GCV in surface water plus sediment extracts decreased rapidly in both systems, from >99% on day 0 to ≤5% in Calwych Abbey and to <5% in Swiss Lake on day 100. A number of unknown but minor (<<10%) transformation products were detected. Two major transformation products, TP1 and TP4, were present in both water and the sediment extracts. These increased in the total water/sediment systems to a maximum level of 13.5% on day 30 in Calwych Abbey before decreasing to 10% on day 62 for TP1 in Calwych Abbey and to 10% on day 62 for TP4 in Calwych Abbey; in Swiss Lake, both TP1 and TP4 remained below 10% throughout. By LC–MS/MS accurate mass analysis, TP1 was tentatively identified as GCV acid (no CAS match found on SciFinder [27]) and TP4 as 9-carboxymethoxymethylguanine (CAS no. 80685–22–9, acyclovir acid) [27]. Because no analytical reference standard was available for either of these transformation products, formally the determinations remain provisional [9]. However, they do allow a proposal of a partial degradation pathway for GCV (Figure 3). In conclusion, GCV is transformed in water/sediment systems over 100 d to a very high degree, with DT50s for the water compartment of 8 d to 10 d and for the sediment of 27 d to 35 d at 20 °C; extrapolating the DT50s to 12 °C shows that they do not meet the European Union criteria for persistence, and hence, despite ready biodegradability not being achieved [3], GCV is not persistent in the environment. Significant to far-reaching \(^{14}CO_2\) production was registered, that is, mineralization in view of the \(^{14}C\) radiolabel on the pyrimidine ring, and a provisional, partial degradation pathway could be drawn.

The OECD guideline 106 adsorption/desorption definitive test with 2 soils, 2 sediments, and 2 activated sludges [11] was performed at a substrate-to-aqueous-phase ratio of 1:1 (w/v) with a 24-h adsorption equilibrium period and a 24-h desorption equilibrium period, based on the results of the preliminary tests. Total recoveries of applied radioactivity were in the range of 86% to 93%, establishing the validity of the assay. For adsorption to soils, \( K_d \) values were 1.23 (Empingham soil) and 0.95 (Warsop soil) L/kg, \( K_f \) values were 1.21 (Empingham soil) and 0.87 (Warsop soil) L/kg, and \( K_{oc} \) values were 34.1 (Empingham soil) and 135 (Warsop soil) L/kg; for soil...
transformation products (TPs) 1 and 4 were tentatively identified based on liquid chromatography–tandem mass spectrometry accurate mass as ganciclovir acid and acyclovir acid, respectively.

Desorption, \( K_d \) values were 2.12 (Empingham soil) and 1.12 (Warsop soil) L/kg, \( K_f \) values were 1.66 (Empingham soil) and 1.03 (Warsop soil) L/kg, and \( K_{OC} \) values were 5.1 (Empingham soil) and 160 (Warsop soil) L/kg. For adsorption to sediments, \( K_d \) values were 1.70 (Calwych Abbey) and 0.78 (Swiss Lake) L/kg, \( K_f \) values were 2.95 (Calwych Abbey) and 0.66 (Swiss Lake) L/kg, and \( K_{OC} \) values were 34.5 (Calwych Abbey) and 131 (Swiss Lake) L/kg; for desorption to sediments, \( K_d \) values were 2.31 (Calwych Abbey) and 0.94 (Warsop soil) L/kg, \( K_f \) values were 3.31 (Calwych Abbey) and 0.82 (Swiss Lake) L/kg, and \( K_{OC} \) values were 47.2 (Calwych Abbey) and 156 (Swiss Lake) L/kg. Desorption values were consistently higher than corresponding adsorption values, indicating that the adsorption was not fully reversible. For both activated sludges, reliable adsorption coefficients \( K_d \) and \( K_{OC} \) could not be calculated, as the adsorption to sludge was low (<7% at 100 g/L), and \( K_f \) and \( K_{OC} \) values were <31 and <100 L/kg, respectively [11]. In conclusion, with the highest \( K_{OC} \) of 160 L/kg in the OECD 106 test, GCV does not sorb strongly to soils, sediments, or activated sludges. Based on the adsorption and desorption constants reported above, GCV can be classified as very mobile in soil and sediment according to the criteria of Briggs [28] and as belonging to the very high to high mobility class according to McCauley et al. [29]. Specifically, the low sorption to activated sludge (\( K_{OC} <100 \) L/kg) means that GCV will not be significantly transferred to the terrestrial compartment through use of surplus sludge for soil amendment. Therefore, soils do not constitute a relevant environmental compartment for GCV and will not be considered further.

Use-based PECs

Based on the VGCV plus GCV use data for the years 2004 to 2014 from IMS Health [12], worst-case total GCV PECs in Europe were calculated. The administered amount of GCV plus the stoichiometric fraction of GCV excreted from VGCV administration served as a basis to calculate a use-based surface water PEC for total GCV, as described in the Materials and Methods section. Note that this PEC does not incorporate any removal during sewage treatment or through partitioning, physicochemical degradation, or biodegradation after the treated wastewater has been released to the environment. For the ERA, the highest European PEC for France in the year 2012 will be used. This worst-case PECsurface water is 0.0141 \( \mu \)g GCV/L, so correspondingly the PECwastewater treatment is 0.141 \( \mu \)g GCV/L, the PECgroundwater is 0.00353 \( \mu \)g GCV/L, and the PECsediment is 0.000 0601 \( \mu \)g GCV/kg dry sediment.

Environmental toxicity and PNECs

The OECD guideline 209 activated sludge test [16] met all the guideline validity criteria: 1) blank control respiration rate \( \geq 20 \text{ mg } \text{O}_2/\text{g} \text{ h} \) was surpassed by a measured 39.4 mg \( \text{O}_2/\text{g} \text{ h} \); 2) the coefficient of variation of the blank control respiration rates was \( \leq 30\% \) as requested; 3) the range for the median effective concentration (EC50) values of the reference substance of 2 mg/L to 25 mg/L for total respiration was met with a measured 11.76 mg/L; and 4) the range for the EC50 values of 3,5-dichlorophenol of 0.1 mg/L to 10 mg/L for nitrification respiration was fulfilled with a measured 4.5 mg/L [16]. Therefore, the test is valid. The definitive test results showed no significant inhibition of heterotrophic, nitrification, or total respiration at the highest tested concentration of 1000 mg/L. On the contrary, the respiration at 1000 mg GCV/L nominal concentration was consistently higher than in the controls [16]. Thus, the NOEC for GCV was 1000 mg/L nominal concentration. Based on this OECD guideline 209 NOEC and using an assessment factor of 10 [14], the PNECwastewater treatment is 100 mg GCV/L.

The definitive OECD guideline 201 algal growth inhibition test was conducted at a single nominal concentration of 100 mg/L, based on a range-finding pretest, plus medium controls [17]. All validity criteria were fulfilled: 1) a requested increase in control cell density by at least a factor of 16 over the 72 h test duration was met with a factor of 158; 2) the mean coefficient of variation for section-by-section specific growth rates (0–24 h, 24–48 h, and 48–72 h into the test) in the control culture of a maximum of 35% was not exceeded by the measured 2.30%.

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Table 1. Dissipation times (DTs) for the different compartments at 20 °C and extrapolated to 12 °C and persistence considerations*

| System/compartment | DT50, d (20 °C) | DT90, d (20 °C) | DT50, d extrapolated to 12 °C | EU persistence \( \% \) criterion, d (12 °C) | Persistent according to EU criteria? |
|---------------------|---------------|---------------|-------------------------------|----------------------------------|----------------------------------|
| Calwych Abbey Lake, total system | 14 | 46 | — | NA | — |
| Calwych Abbey Lake, water | 8 | 26 | 15.2 | 40 | No |
| Calwych Abbey Lake, sediment | 27 | 91 | 51.2 | 120 | No |
| Swiss Lake, total system | 18 | 82 | — | NA | — |
| Swiss Lake, water | 10 | 51 | 19.0 | 40 | No |
| Swiss Lake, sediment | 35 | (117) | 66.4 | 120 | No |

*The DT50 and DT90 values refer solely to quantified ganciclovir, but not to total radioactive residues in the test systems. The extrapolated DT50 at 12 °C was calculated using the following equation: \( DT50(12 \text{ °C}) = DT50(20 \text{ °C}) \times e^{0.08[20-12]} = DT50(20 \text{ °C}) \times 1.8965 \) [26]. Persistence criteria for water and sediment according to the REACH Guidance [25]. The DT90 value in parentheses is extrapolated beyond the duration of the test. DT50 = 50% dissipation time; DT90 = 90% dissipation time; EU = European Union; NA = not applicable; \( \% \) = half-life.

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Figure 3. Proposed partial degradation pathway for ganciclovir. The major transformation products (TPs) 1 and 4 were tentatively identified based on liquid chromatography–tandem mass spectrometry accurate mass as ganciclovir acid and acyclovir acid, respectively.
and 3) the coefficient of variation for average specific growth rates during the whole test in replicate control cultures of a maximum of 7% was not exceeded by the measured 1.69% [17].

Analysis of test media samples conducted at 0 h and 72 h resulted in measured GCV concentrations of 111 mg/L and 106 mg/L, respectively. Based on nominal concentrations, the 72-h EC50 for the endpoint yield and the 0-h to 72-h EC50 for biomass and growth rate were estimated to be greater than 100 mg/L (109 mg/L based on mean measured concentration), while the corresponding NOEC values for yield, biomass, and specific growth rate after 72 h were 100 mg/L (109 mg/L mean measured concentration), the highest tested concentration [17].

The OECD guideline 211 daphnid reproduction assay was conducted at nominal concentrations of 0.032, 0.1, 0.32, 1.0, and 3.2 mg GCV/L, based on a range-finding pretest, plus medium controls [18]. All validity criteria were fulfilled: 1) mortality of parental *D. magna* in the control treatment, stipulated as ≤20% in the OECD guideline 211 test, was 0% by the end of the test; and 2) the mean number of live offspring produced per parental *D. magna* (female) surviving at the end of the test, requested to be >60, was 106 [18]. Chemical analysis of the test preparations showed that most of the samples were within the acceptance limits of 80% to 120% of the nominal concentration, with a range of 82% to 116% of the nominal concentration; on 5 occasions results in excess of 120% of the nominal concentration were observed, but these values were not considered to affect the integrity of the test, given that the majority were above the NOEC. In comparison with the control group, there were no statistically significant effects on adult survival, total number of juveniles produced per surviving adult, adult growth (based on final length), or intrinsic rate of population increase at any of the concentrations employed. Thus, the overall NOEC, based on nominal concentrations, was the highest tested concentration of 3.2 mg/L nominal concentration, corresponding to 3.3 mg/L mean measured concentration, also the highest tested concentration [18]. The PNEC<sub>groundwater</sub>, derived from the daphnid chronic concentration was less than 10%; therefore the EC10 value was calculated, which was estimated to be 0.067 mg/L. As the EC10 value can be considered to be the value where no biologically significant effects are observed, the NOEC for length and weight of the F1 generation was set at 0.012 mg/L mean measured concentration [19].

The OECD guideline 229/210 fish partial life cycle test was conducted at nominal concentrations of 0.010, 0.10 and 1.0 mg GCV/L, based on a range-finding pretest, plus medium controls [19]. Most validity criteria were fulfilled: 1) the control mortality in the F0 generation, which should not exceed 10% at the end of the 21-d exposure period, was 1 male and 1 female out of 24 control fish; 2) the dissolved oxygen concentration, required to be at least 60% of the air saturation value for the whole duration of the test, showed a lowest measured value of 93%; 3) the water temperature should not differ by more than 1.5 °C between test chambers at any time during the test (this criterion was not met in replicate 1 in the control group, which varied by 1.6 °C; but because no adverse effects were observed in the replicate and the temperature did not vary by more than 1.5 °C between consecutive readings, this deviation was considered not to affect the integrity of the test); 4) hatching success in the control treatments of the F1 generation, stipulated to be ≥70%, was 76%; and 5) posthatch survival of fish larvae in the control treatments of the F1 generation, required to be ≥75%, was 76% [19]. In general, measured concentrations were within 80% to 120% of the nominal concentration, but some of the determinations were slightly in excess of the 120% of the nominal concentration. Given that some of the measured concentrations were outside the expected range, the results were calculated based on mean measured concentrations, which were 0.012, 0.12, and 1.1 mg/L [19].

For the OECD guideline 229 reproduction phase of the test, 2 of 24 control fish but none of the 24 exposed F0 fish in total per concentration died during the duration of 21 d. In addition, no abnormal changes in body color, body shape, or territorial aggressiveness were observed in any of the test groups compared with the control. Statistical analysis showed no significant difference (*p* < 0.05) at any of the test concentrations compared with the control in terms of the number of spawns or eggs produced; specifically, the mean number of spawns per surviving female was 2 for the controls and 3 for 0.01 mg/L nominal concentration, 2 for 0.10 mg/L nominal concentration, and 3 for 1 mg/L nominal concentration, whereas the mean number of eggs per surviving female was 212 for the controls and 268 for 0.01 mg/L nominal concentration, 229 for 0.10 mg/L nominal concentration, and 322 for 1 mg/L nominal concentration. Furthermore, there were no statistically significant effects (*p* < 0.05) on either fish length or fish weight at any of the concentrations employed in the test compared with the control for either male or female fish. In addition, no differences were observed between the control and test concentrations in terms of phenotypic sex for all F0 fish in the test, and all female fish showed the presence of eggs. Lastly, statistical analysis showed no significant difference (*p* < 0.05) at any of the test concentrations compared with the control in terms of vitellogenin concentration for either male or female fish. Therefore, the NOEC for F0 reproduction based on parental mortality, mean number of spawns and of eggs, length and body mass, phenotypic sex, and egg production as well as sex-dependent vitellogenin concentration of surviving F0 fish was 1.1 mg/L mean measured concentration [19].

For the OECD guideline 210 early life stage development phase of the test, the first egg hatch in all treatment and the control vessels occurred between days 3 and 5 post egg addition. This indicated no difference in the time to first hatch across all treatments compared with the control groups. The mean hatching success of embryos in the control group was 76%, while in the treatments it ranged between 90% and 96%; there were no statistically significant (*p* < 0.05) effects on hatching success at any of the exposure concentrations compared with the controls. The mean posthatch survival in the control group was 76%, while the mean posthatch survival in the treatments ranged between 65% and 84%; there were no statistically significant effects on posthatch survival at any of the test concentrations used compared with the control. In contrast, the measurements of total length (mm) and wet weight (mg) of the single surviving F1 fish at the end of the test phase (day 35) showed significant effects (*p* ≥ 0.05) on weight at the 0.10 mg/L and 1.0 mg/L nominal concentrations, with both weight and length being reduced compared with the controls. Statistical analysis of the length data showed significant differences (*p* ≥ 0.05) between all test concentrations and the control. The observed effect at the lowest exposure concentration of 0.01 mg/L nominal concentration was less than 10%; therefore the EC10 value was calculated, which was estimated to be 0.067 mg/L. As the EC10 value can be considered to be the value where no biologically significant effects are observed, the NOEC for length and weight of the F1 generation was set at 0.012 mg/L mean measured concentration. Lastly, no significant developmental abnormalities were observed for the F1 fish in the controls and all test concentrations throughout the duration of the test [19]. Therefore, the NOEC for the OECD guideline 210 phase and also for the whole OECD guideline 229/210 partial life cycle test in fathead minnows was 0.012 mg GCV/L mean measured concentration [20].
concentration, with the F1 generation growth parameters driving this NOEC.

Based on the NOECs for the surface water model organisms algae of 109 mg/L, mean measured concentration [17], daphnids of 3.3 mg/L, mean measured concentration [18], and fish of 0.012 mg/L, mean measured concentration [19], the PNEC_{surface water} is 0.0012 mg GCV/L or 1.2 μg GCV/L, using an assessment factor of 10.

The OECD guideline 218 chironomid development and emergence test NOEC was conducted at nominal concentrations of 0.32, 1.0, 3.2, 10, 32 and 100 mg GCV/kg dry sediment, based on a range-finding pretest, plus medium controls [20]. Most validity criteria were fulfilled: 1) adult midge emergence from the control treatment, which must be at least 70% at the end of the test, was 86%; 2) ideally, adult emergence from control vessels, which should occur between days 12 and 23 after larval addition to the test system, occurred between days 16 and 22. 3) at the end of the test, the pH in the overlying water in all test vessels, which should be within the range of pH 6 to 9, was between pH 8.43 and 8.65; 4) at the end of the test, the dissolved oxygen in all test vessels, which should be greater than 60% of the air saturation value, was between 93% and 99%; and 5) the water temperature monitored should (ideally) not vary by more than ±1°C over the test duration (this criterion was slightly exceeded by a total temperature range of 20.2°C to 22.6°C; however, in view of the biological results, this deviation was not considered to affect the integrity of the test) [20]. The results of radiochemical analysis of the sediment on day 1 (day of preparation) showed concentrations that were 91% to 100% of target based on total radioactivity, equivalent to 0.33 mg/kg to 92 mg/kg. Analysis of the day 0 samples showed 49% to 79% in the sediment, 8.7% to 20.2% in the porewater, and 5.7% to 9.8% in the overlying water, indicating that the majority of the test substance was within the sediment/porewater compartment of the test system at the beginning of the test. Analysis of the day 28 samples at 0.32 mg/kg and 100 mg/kg showed 60% and 54%, respectively, to be in the sediment, 4.6% and 5.7% in the porewater, and 25% and 32% in the overlying water, indicating partitioning of the test substance to the overlying water layer over the 28-d test period. Analysis by LC–MS/MS of the 100 mg/kg nominal concentration sediment extract on day 0 showed that 99% of the detected radioactivity was attributable to the parent test substance, while on day 28, 90% of the detected radioactivity was attributable to GCV, indicating that no significant degradation of GCV occurred in the test system over the duration of the test. The first emergence of adult chironomids from each test vessel occurred between days 14 and 18. There was no statistically significant difference (Chi-squared test, p ≥ 0.05) between male and female emergence or on the sex ratio, and therefore the data were pooled for further considerations. Statistical analysis of the emergence data and development rates showed no significant differences (p ≥ 0.05) between the test groups and the control. The NOEC value for emergence and development rate was therefore 100 mg/kg nominal concentration, corresponding to 79 mg GCV/kg dry weight highest tested concentration initial measured concentration. Based on the latter initial concentration and on an organic carbon content of 2.4% in the artificial sediment, the 10% organic carbon–normalized NOEC [23] is 329.2 mg GCV/kg dry weight. Dividing this figure by an assessment factor of 100 in view of 1 available chronic sediment toxicity test, the PNEC_{sediment} is 3.292 mg GCV/kg [14].

An MTDI for GCV for (semi)aquatic top predators is derived [22] based on available acute and (sub)chronic mammalian toxicity data [4]; although acute tests were performed with oral dosing, most of the (sub)chronic assays were performed with intravenous administration in view of the low oral bioavailability of GCV. Specifically, the acute median lethal doses (LC50s) were higher than the highest dose of 2000 mg/kg body weight in mice and of 1000 mg/kg body weight in dogs. In the (sub)chronic tests, the lowest no-observed-adverse-effect levels for GCV were 1) a daily intravenous dose of 6 mg/kg body weight in a developmental toxicity and teratogenicity study in the rabbit; 2) a daily intravenous dose of 0.4 mg/kg body weight in a fertility and reproductive performance study in mice, where the adverse effect at higher doses was testicular toxicity; 3) a daily oral dose of 1 mg/kg body weight in an 18-mo carcinogenicity study in mice; and 4) a daily intravenous dose of 0.06 mg/kg body weight in a 12-mo chronic toxicity study in dogs, where the only effect was sebaceous gland atrophy [4]. Dividing the lowest LC50 of >1000 mg/kg by an assessment factor of 100 [22] results in an acute-based MTDI of >10 mg/kg body weight/d, whereas dividing the lowest chronic or reprotoxicity no-observed-adverse-effect level of 0.06 mg/kg body weight/d by an assessment factor of 30 [22] gives a chronic-based MTDI of 0.002 mg/kg body weight/d. The latter, lower MTDI was selected for top predator risk assessment.

**Environmental risk assessment**

Based on the PECs and PNECs derived, potential risks for the environmental compartments wastewater treatment, surface water, groundwater, and sediment can be expressed as the risk characterization ratio of PEC divided by PNEC. A risk characterization ratio of ≥1 is generally seen as an indication for potential risk, while a risk characterization ratio of <1 suggests no significant risk based on the available data. The PEC and PNEC values, and the corresponding risk characterization ratios for the above compartments, are shown in Table 2. For the wastewater treatment the risk characterization ratio is 1.41 × 10^{-6}, for surface freshwaters it is 1.18 × 10^{-2}, for groundwater it is 1.07 × 10^{-5}, and for sediments it is 1.83 × 10^{-5}. The risk characterization ratios reveal that for all of the relevant environmental compartments for GCV, no significant risk is identified from the current use of VGCV and GCV.

Regarding (semi)aquatic top predators, based on the above MTDI, an otter of 10 kg body weight [22] may consume up to 0.02 mg GCV/d (10 kg body wt × 0.002 mg/kg body wt/d) from the daily consumption of 1 kg fish and 0.79 L surface water [22] without incurring any untoward risks from GCV. The GCV concentration in fish is predicted as the BCF of GCV multiplied by the PEC_{surface water} [22]. Because GCV is not expected to bioconcentrate based on its negative log Kw, and as no empirical BCF data exist, a modeled BCF must be used: SciFinder models a BCF of 1 L/kg over the whole pH range of 1 to 10 [27], whereas EPISuite calculates a BCF of 3.16 L/kg [30]. Therefore, a fish of 1 kg body weight is predicted to contain 1 kg × 0.0141 μg GCV/L × 3.16 L/kg, or 0.0446 μg GCV; furthermore, 0.79 L of surface water contains 0.79 L × 0.0141 μg GCV/L, or 0.0111 μg GCV. Both sources together add up to 0.0557 μg GCV/d. This is far below the MTDI for an otter, resulting in a risk characterization ratio of 2.78 × 10^{-3}; hence, no risk is predicted for top predators.

For human exposure from drinking water and fish consumption, the same principles apply; a daily drinking water consumption of 2 L and a daily fish consumption of 0.115 kg is assumed [15,22]. Assuming no removal of GCV during drinking water treatment, 2 L of drinking water contain...
2 L × 0.0141 µg GCV/L, or 0.0282 µg GCV; an average daily fish ration contains 0.115 kg × 0.0141 µg GCV/L × 3.16 L/kg, or 0.00512 µg GCV. When these values are taken together, the predicted daily GCV uptake is 0.0333 µg GCV. For humans, there is an acceptable daily exposure value for oral uptake of 500 µg GCV for a 60-kg person, which is derived from the same experimental data as above but is higher than that for a top predator because only chronic oral toxicity studies were used (and indeed, inhalative acceptable daily exposure values are lower) [4]. Comparing the predicted daily uptake with the acceptable daily exposure results in a risk characterization ratio of 6.66 × 10⁻⁴ (Table 2); hence, no risk is foreseen from human indirect exposure. Note that because top predators are also exclusively exposed through oral uptake, it would be legitimate to use the human acceptable daily exposure value for the otters as well, which would lower the top predator risk characterization ratio.

### Table 2. Predicted environmental concentrations (PECs) or exposure, predicted no-effect concentrations (PNECs) or safe levels, and PEC/PNEC risk characterization ratios (RCRs) for total ganciclovir (GCV) in Europe based on highest actual per capita use of valganciclovir and ganciclovir in the years 2004 to 2014

| Environmental compartment/receptor | PEC/exposure | PNEC/safe level | RCR (PEC/PNEC) | Risk? |
|-----------------------------------|--------------|----------------|----------------|------|
| Wastewater treatment, (µg GCV/L)  | 0.141        | 100 000        | 0.000000141    | No   |
| Surface freshwaters (µg GCV/L)   | 0.0141       | 1.2            | 0.0118         | No   |
| Groundwater (µg GCV/L)           | 0.00353      | 330            | 0.0000107      | No   |
| Sediments (µg GCV/kg)            | 0.0601       | 3292           | 0.0000183      | No   |
| (Semi)aquatic top predator (otter) (µg GCV/d) | 0.0557 | 20 | 0.00278 | No |
| Human indirect exposure (µg GCV/d)| 0.0333       | 500            | 0.0000666      | No   |

Environmental PBT assessment

The OECD guideline 308 sediment/water transformation test showed that GCV undergoes rapid transformation and biodegradation at 20 °C [9], so that even after normalizing the DT50 values for the water and sediment compartments to a temperature of 12 °C [26], the European Union criteria for persistence [25] are not met (Table 1). Therefore, despite not meeting the screening criteria in laboratory biodegradation tests [3], GCV is not persistent.

Based on a measured log $K_{OW}$ of −1.95 at pH 5 and 7 and of −2.0 at pH 9 [3], GCV is far from the European Union screening criterion for bioaccumulative of log $K_{OW}$ ≥ 3 [14, 25]. Without experimental bioconcentration data, quantitative structure–property modeling suggests BCFs for GCV of 1 [27] and 3.16 [30], which are both far below the European Union bioaccumulative criterion of a BCF of 2000 [25]. Hence, based on the European Union screening criterion and on 2 commonly used models, GCV is not bioaccumulative.

Based on mammalian CMR properties [4], GCV is formally toxic; but the European Union environmental toxic criterion is a chronic aquatic ecotoxicity NOEC or EC10 of ≤0.01 mg/L [25]. In the algal, daphnid, and fish partial life cycle tests with GCV presented in the present study [17–19], the lowest NOEC was 0.012 mg/L mean measured concentration in the fish test, whereas the EC10 in this test was 0.067 mg/L [19]. Specifically, there was no indication of mutagenicity in the rapidly dividing algae [17], in the daphnid parthenogenetic reproduction [18], or in the fish gametogenesis [19]. The development of algae and daphnids was unaffected; in fish there were no adverse effects on parental fertility or F1 embryonic development or teratogenicity, but there was some F1 growth inhibition [19]. Therefore, while GCV is toxic based on mammalian toxicity, it is not toxic based on chronic aquatic toxicity; moreover, it is neither persistent nor bioaccumulative. As all 3 criteria must be met, GCV is not PBT.

### DISCUSSION

Ganciclovir and subsequently VGCV were developed as antivirals for the treatment and prevention of cytomegalovirus and other herpesviruses infections and indeed VGCV is on the World Health Organization list of essential medicines [31], emphasizing its global importance. Like other antivirals, the actual API GCV is a purine base (specifically a guanine) analogue, which may explain its mutagenic and reprotoxic properties in mammals. These very properties, requiring a CMR classification in Europe, led to the necessity for an updated ERA based on new environmental fate and effects tests, with special consideration of reprotoxic and, indirectly, mutagenic properties in aquatic organisms. This update has resulted in 2 major new findings. First, even though GCV is not biodegradable in a laboratory test [3], thereby matching an European Union screening criterion for persistence [25], an OECD guideline 308 sediment/water environmental fate test showed that it was widely transformed and biodegraded; even with the DT50 values recalculated for 12 °C, the definitive criteria for persistence in water and sediment [25] are not met, and hence GCV is not persistent. Second, the new chronic ecotoxicity tests with algae, daphnia, and fish [17–19] showed no adverse effects on the algae and daphnids at the highest tested concentrations [17, 18]. In the fish test, obvious impacts were noted, but these did not affect fish gametogenesis, fertilization, or embryonic development with meiotic and mitotic cell divisions, nor did they affect the potential for malformations, where mutagenic or general reprotoxic effects might be expected to show up. The inhibitory effects of GCV in the fish partial life cycle test manifested as a growth inhibition measured by the endpoints length and mass of the F1 fish [19]. While this is recognized to be a significant effect, it does not suggest mutagenicity or teratogenicity. In support of this hypothesis, a chironomid growth and emergence test also did not show any adverse effects of GCV up to the highest tested concentration compared with the controls [20]. Also, an old 1991 microbial growth inhibition test following the 1987 US Food and Drug Administration Technical Assistance Document 4.02 [32], investigating the growth of bacteria (Clostridium perfringens, Streptomyces fragmentans spp. aquaticus), fungi (Aspergillus niger, Trichoderma viride), and cyanobacteria (Nostoc sp.) on agar plates, showed no inhibitory effects on any of the 5 microorganisms at a highest tested concentration of 1000 mg GCV/L [3], supporting the new activated sludge respiration inhibition test results [16]. The PNECs derived in the present study for various environmental compartments and receptors may be used for ERAs of GCV and VGCV in other parts of the world.
Valganciclovir is hydrolyzed by enteric and hepatic esterases to GCV; but thanks to the strongly improved bioavailability, VGCV has a defined daily dose of only 0.9 g [38], of which only 72% by mass or 0.65 g/defined daily dose is GCV, the remainder corresponding to the L-valine moiety. All GCV is essentially excreted unchanged [4]. Hence, the complete replacement of GCV by VGCV would result in a very strong reduction of total GCV excretion per patient from originally 100% to 22% (0.65 g/d ÷ 3 g/d), while fully maintaining therapeutic levels of the active substance GCV. This corresponds to a decrease in environmental exposure of GCV of nearly 80% per patient, without any loss in efficacy [39]. The time span from 2004 to 2014 saw the above increase in actual VGCV use from approximately 1 056 000 to 3 333 000 defined daily doses and a decrease in GCV from approximately 200 000 to 53 000 defined daily doses, which results in a combined increase in defined daily doses from 1 256 000 to 3 280 000 (i.e., by a factor of roughly 2.6). Over the same time period, total annual GCV excretion, based on combined VGCV and GCV administered, increased from approximately 1286 kg to 2327 kg (i.e., by a factor of 1.8). Although the number of patients receiving VGCV or GCV nearly doubled, the actual excretion of GCV per patient has decreased to 69.3% of the 2004 value; even in 2004, VGCV already had a substantial share in the combined use. Therefore, since the introduction of VGCV in 2001, the far-reaching replacement of GCV by VGCV has resulted in a massive decrease in environmental exposure by GCV, despite a marked increase in patients receiving the drugs.

CONCLUSIONS

Even though GCV is not readily biodegradable, suggesting limited removal in sewage works, it proved to be nonpersistent in a sediment/water environmental fate test. Ganciclovir is not persistent and not bioaccumulative; and while it is toxic based on mammalian data, it is not toxic based on chronic aquatic ecotoxicity; therefore, GCV is not PBT. Specifically, chronic algal, daphnid, and the fish partial life cycle tests showed no mutagenic, teratogenic, or developmental adverse effects apart from growth inhibition in the fish F1 generation. The risk characterization ratios for GCV based on the documented combined use of VGCV and GCV for wastewater treatment, surface water, groundwater, and sediment consistently showed no significant risk; soil is not a relevant compartment for GCV. Similarly, GCV poses no significant risk to (semi)aquatic top predators or to human consumers of water and fish. Therefore, at current use levels (and also allowing for an increase in use), the medicinal use of GCV and VGCV does not pose a significant risk to the aquatic environment.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3758.

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Conflict of Interest—The author is a full-time employee of the pharmaceuticals and diagnostics company F. Hoffmann-La Roche in Basle,
Switzerland, where he works as the company’s Environmental Risk Assessor.

Data Availability—The basic data are either GLP test reports from a contracted laboratory paid for by Roche or data belonging to IMS Health on use of the 2 pharmaceuticals, for which permission was received to cite the data but not to pass them on. Anyone may come to Basle in person and review the original reports, without copying, photographing (including smartphone), or transmitting them electronically.

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