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Revolade® (eltrombopag olamine)

12.5, 25, 50 and 75 mg film-coated tablets and 25 mg powder for oral suspension (PfOS)

Severe aplastic anemia (SAA)

1.6.1 Environmental Risk Assessment Non-GMO

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List of abbreviations

API	Active Pharmaceutical Ingredient
CHMP	Committee for Medicinal Products for Human Use
$DOSE_{ai}$	Maximum Daily Dose of Active Substance Consumed per Inhabitant
EMEA	European Agency for the Evaluation of Medicinal Products
ERA	Environmental Risk Assessment
EU-CONai	Maximum Annual Amount of Active Ingredient Consumed in the EU
F_{pen}	Penetration Factor
K_{oc}	Organic Carbon Normalised Adsorption Coefficient
K_{ow}	Octanol/Water Partition Coefficient
NOEC	No Observed Effect Concentration
OECD	Organisation for Economic Co-operation and Development
PBT	Persistent, Bioaccumulative and Toxic
PEC	Predicted Environmental Concentration
PNEC	Predicted No-Effect Concentration
SAA	Severe Aplastic Anemia
STP	Sewage Treatment Plant
WASTEW	Wastewater

Signatures

Author

[redacted]

Global Pharma Environment

Signature	Date

1 Executive summary

The Environmental Risk Assessment (ERA) for Revolade® (eltrombopag olamine) 12.5, 25, 50 and 75 mg film coated tablets (referred to in this document as eltrombopag tablets) and 25 mg Powder for Oral Suspension (PfOS), is required for both the product and packaging by Directive 2001/83/EC, as amended. Each strength of eltrombopag tablet contains 25, 50 or 75 mg of eltrombopag and the PfOS contains 25 mg eltrombopag olamine (also called by the GSK code number SB-497115-GR), the bis-monoethanolamine salt of the free acid eltrombopag.

Eltrombopag is a small molecule, non-peptide, orally active thrombopoietin receptor (TPO-R) agonist that functions in a similar manner to endogenous thrombopoietin (TPO) the main cytokine involved in regulation of megakaryopoiesis and platelet production.

Eltrombopag was first registered in the EU in March 2010 for use in the treatment of adult chronic immune (idiopathic) thrombocytopenic purpura (cITP) splenectomised patients who are refractory to other treatments (e.g. corticosteroids, immunoglobulins) [EMEA/H/C/001110]. Eltrombopag tablets may be considered as second line treatment for adult non-splenectomised patients where surgery is contraindicated. Subsequent variations to eltrombopag have been submitted and approved for usage in chronic hepatitis C (cHCVaT) infection support [EMA/H/C/001110/X/0012G]; severe aplastic anaemia (SAA) which is a rare, life-threatening, acquired bone marrow failure disease [EMEA/H/C/001110/II/0020]; and for an extension for the treatment of ITP in non-splenectomized patients [EMEA/H/C/001110/II/0023]. Further, the marketing application holder (MAH) completed development of paediatric studies for use in the treatment of pediatric patients (children age 1 year and above) with ITP submitted under Article 46 which included the development of a lower strength tablet (12.5 mg) and the PfOS formulation [EMEA/H/C/001110/X/0022/G]. All indications approved to date are treatments of rare diseases.

The original ERA for eltrombopag identified a measured partition coefficient for this molecule, at pH 7, of greater than 4.5 and, on the basis of this observation and as a part of the approval process, the MAH agreed to undertake, as a follow-up measure, a staged Persistence, Bio-accumulation and Toxicity (PBT) assessment of eltrombopag in a stepwise manner in accordance with EU REACH guidelines (FUM001). Accordingly, a Bioconcentration: Flow-through fish test (OECD 305) has been performed. An updated version of the original ERA on the impact of eltrombopag tablets on the environment was submitted, together with a discussion as to how the new information informed the need for any further PBT assessment with eltrombopag, in November 2011.

The present application addresses the following new indication:

Revolade is indicated in combination with standard immunosuppressive therapy for the first-line treatment of adult and pediatric patients 2 years and older with severe aplastic anemia.

The maximum recommended dose for SAA is 150 mg once daily.

This document provides an updated environmental assessment of eltrombopag tablets and PfOS product.

The following conclusions are made:

According to current guidance, the fate of only one component of the product, including packaging, requires consideration. This component is the drug substance, eltrombopag.

The remaining components of the eltrombopag tablets or the eltrombopag PfOS product, including packaging, are already introduced into the environment from a variety of sources in much greater quantity. Therefore, introducing these components into the environment as a result of the use of this product is not expected to result in adverse environmental effects.

Using the Guidance on "Environmental Risk Assessment of Medicinal Products for Human Use" (EMEA/CHMP/SWP/4447/00 corr 2) a Phase 1 calculation for prediction of PEC has been made and the calculated PEC for eltrombopag (0.75 µg/L) was above the trigger value of 0.01 µg/L. Thus, a full Phase II Tier A assessment has been carried out and extended fate and effects analysis has been conducted as part of Tier B.

Eltrombopag is not readily nor inherently biodegradable and is not expected to be extensively mineralized (converted to CO_2). No significant removal of the parent by primary degradation is expected at the sewage treatment plant (STP).

Eltrombopag is not water soluble but is slightly water soluble at basic pH. This substance is moderately lipophilic at neutral pH, and the water/sludge distribution coefficient (Koc) suggests that in the aquatic environment it is expected to sorb to suspended particles and sediment to a significant extent. Although this substance is considered to be persistent it is expected to be relatively immobile in the aquatic environment.

The adsorption potential of eltrombopag has been investigated in a batch-equilibrium study with two sludges and three soils. The results of this study indicate that the adsorption potential of eltrombopag in sludges remains below the trigger value, i.e. Koc < 10'000 and consequently no risk assessment is required for the terrestrial environment.

This substance is not toxic to activated sludge microbial populations. In chronic studies this substance exhibits toxicity to algae, daphnia and fish. However, it should be noted that surface water concentrations are unlikely to reach a level where effects are expected to be observed.

Eltrombopag was shown not to significantly accumulate in fish tissue at test concentrations between 5 and 50 times the predicted "worst case" PEC for eltrombopag. In this study, at the end of the 10 day depuration period, 88% and 85% of eltrombopag olamine was eliminated from the fish tissues at each concentration, respectively. Based on this information the biological half-life for eltrombopag is considered to be between 3 and 10 days. Thus, eltrombopag does not fulfil the criteria for bioaccumulation as defined in the EU REACH Testing Guidelines and therefore it can be concluded that eltrombopag does not fulfil the criteria to be considered a PBT/vPvB (i.e. Persistent/Bioaccumulative/Toxic or very Persistent/very Bioaccumulative) substance as set out in the guidance.

The Phase II Tier A $PEC_{SURFACEWATER}$ has been calculated using epidemiological data for all currently registered and newly applied for indications, i.e. cHCVaT, SAA, cITP and first line SAA (total PEC) based on the assumption that 100% of drug substance used enters the sewage treatment plant unchanged and passes through into the aquatic environment.

This ERA concludes that eltrombopag is unlikely to represent a risk to the aquatic environment.

A Phase II Tier B risk assessment has been conducted for sediment compartments, based on a toxicity study in sediment-dwelling larvae of the non-biting midge *Chironomus riparius*. The PEC for sediment compartments has been based on a refined $PEC_{surface\ water}$ using epidemiological information on all indications and SimpleTreat modelling. The risk ratio for sediment compartments indicates no immediate risk.

Following use of eltrombopag tablets, all components derived from the drug substance will be released into waste water systems.

Adverse environmental effects are not expected to occur as a result of emissions associated with the disposal of returned unused eltrombopag tablets product, including the packaging components when disposed of in accordance to instructions at suitable licensed facilities.

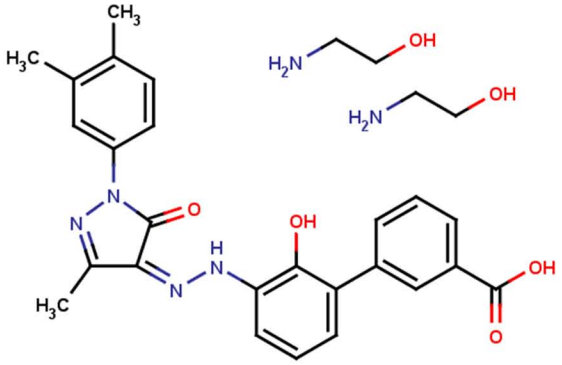
2 Introduction

Eltrombopag is a small molecule, non-peptide, orally active thrombopoietin receptor (TPO-R) agonist that functions in a similar manner to endogenous thrombopoietin (TPO) the main cytokine involved in regulation of megakaryopoiesis and platelet production.

In clinical studies, following oral administration, most of the eltrombopag dose was recovered in urine and faeces by 144 hours post dose (see m2.7.2, section 2.1.1 of the original MAA). Overall, 90% of the dose was recovered in the excreta; 59% of the dose was recovered in faeces and 31% was recovered in urine. Absorbed eltrombopag was extensively metabolized. On average, 59% of the dose was recovered in faeces; 20% of the recovered dose was unchanged eltrombopag and 21% was three co-eluting glutathione-related conjugates, including a glutathione, glutamyl-cysteine, and cysteine adduct. On average, 31% of the dose was recovered in urine; none of the recovered dose was unchanged eltrombopag, and 20% was a glucuronide of the phenylpyrazole moiety resulting from hydrazine cleavage of eltrombopag. NMR analysis confirmed that a glucuronide conjugate of the biphenyl moiety (unlabeled portion) resulting from hydrazine cleavage of eltrombopag was also present in urine in similar proportions to that of the phenylpyrazole moiety. The remaining unaccounted dose in the excreta was comprised of multiple metabolites that could not be individually quantified because their levels were close to or below background (19% of dose overall; 8% in faeces and 11% in urine), and losses incurred during sample processing and analysis (10% of dose in faeces).

In conclusion, following oral administration of eltrombopag to man, eltrombopag will be released into the environment primarily as metabolites with some parent compound. All human metabolites of eltrombopag are more polar than the parent compound, and thus unlikely to be more environmentally persistent. Therefore, the characteristics of the metabolites of eltrombopag are such that they require no further consideration in this ERA. Thus, the assessment that follows has been produced on the assumption that only the pharmacologically more potent, more lipophilic, parent compound eltrombopag is relevant to this ERA.

Table 2-1 Eltrombopag olamine structure and physico-chemical properties

Chemical structure	
Chemical name	3'-{N'-[1-(3,4-Dimethyl-Phenyl)-3-Methyl-5-Oxo-1,5- Dihydropyrazol-4-Ylidene] Hydrazino}-2'-Hydroxybiph Enyl-3-Carboxylic Acid, Ethanolamine 1:2 Salt
Molecular formula	$C_{25}H_{22}N_4O_4 * 2(C_2H_7NO)$
Relative molecular mass	442.48 (free acid)
	564.65 (olamine salt)
Melting point	244 °C
pK_a	pKa1 = 3.5
	pKa2 = 8.5
UV absorption maxima	248 nm, 417 nm

In addition to the active pharmaceutical ingredient (API), the tablets contain the following excipients which either occur naturally or are not of environmental concern: mannitol, cellulose microcrystalline, Povidone K30, sodium starch glycolate (Type A), magnesium stearate and film coat (Opadry® White YS-1-7706-G, Opadry® Brown 03B26716, Opadry® Pink 03B24157, Opadry® Green 03B21485). The marketing of Revolade does not increase the environmental load of any of these commonly used excipients to a significant extent.

3 Phase I: Environmental exposure assessment

3.1 PEC calculation

The predicted environmental concentration (PEC) is given by the formula proposed in guideline EMEA/CHMP/SWP/4447/00 corr 2 and is calculated as the sum of all PECs for all indications for which eltrombopag is applied:

$PEC_{surface\ water}$ = $(DOSE_{ai} * F_{pen}) / (WASTEW_{inhab} * DILUTION)$

= (75 mg/inhabitant/day * 0.01) / (200 L/inhabitant/day * 10)

= **0.000375 mg/L = 0.375 µg/L for ITP**

$PEC_{surface\ water}$ = $(DOSE_{ai} * F_{pen}) / (WASTEW_{inhab} * DILUTION)$

= (100 mg/inhabitant/day * 0.01) / (200 L/inhabitant/day * 10)

= **0.0005 mg/L = 0.5 µg/L for cHCV associated thrombocytopenia**

$PEC_{surface\ water}$ = ($DOSE_{ai}$ * F_{pen}) / ($WASTEW_{inhab}$ * DILUTION)

= (150 mg/inhabitant/day * 0.01) / (200 L/inhabitant/day * 10)

= **0.00075 mg/L = 0.75 µg/L for SAA**

Overall $PEC_{surface\ water}$ = 0.375 µg/L + 0.5 µg/L + 0.75 µg/L = **1.625 µg/L**

Where:

$DOSE_{ai}$ = 75 mg/patient/day for ITP;

= 100 mg/patient/day for cHCV associated thrombocytopenia

= 150 mg/patient/day for 6 months for SAA

F_{pen} = 1% (default)

$WASTEW_{inhab}$ = 200 L/inhabitant/day

DILUTION = 10

Conclusion: The overall predicted environmental concentration (PEC), taking into account all indications, is 1.625 µg/L, which exceeds the trigger value of 0.01 µg/L. The assessment therefore proceeds to Phase II – Tier A.

4 Phase II – Tier A: Environmental fate and effects analysis

4.1 Initial fate and effects analysis

4.1.1 Physico-chemical, fate and effect studies

Table 4-1 Base data set eltrombopag olamine

Water solubility	0.416 mg/L at pH 7.4
n-Octanol/water partition coefficient	log D_{ow} > 4.1 at pH 5 log D_{ow} = 4.52 at pH 7 log D_{ow} = 0.96 at pH 9 (Report SD2006/02328/00)
Adsorption/Desorption using a batch equilibrium method (OECD 106)	K_{oc} sludge = 8'534 and 7'703 mL/g K_{oc} soil = 155'386, 97'529 and 144'754 mL/g K_d sludge = 2'355 and 2'148 mL/g K_d soil = 1'538, 1'307 and 1'723 mL/g (Report 2014N196411_00)
Inherent Biodegradability (OECD 302C)	Not inherently biodegradable

	Ultimate biodegradation = 14 %, 28 days Primary biodegradation = 10 %, 28 days (Report SD2006/00090/00)
Hydrolysis as a function of pH (OECD 111)	pH 7 $t_{1/2}$ = 16 days (14°C)* pH 9 $t_{1/2}$ = 752 days (14°C) (Report SD2006/03293/00) * Not strongly first order and rates don't scale according to temp
Transformation in Aquatic Sediment Systems (OECD 308)	DT50 (total water and sediment) > 120 days* DT90 (total water and sediment) > 120 days (Report 2014N209349_00) * Data did not allow calculation of a DT50 value per se. This is an extrapolation from a minimum data set.
Lemna Growth Inhibition Test (OECD 221)	Average Specific Growth rate: E_rC_{50} (frond number) = 4.1 mg/L NOEC (frond number) = 0.45 mg/L E_rC_{50} (dry weight) = 7.2 mg/L NOEC (dry weight) = 0.45 mg/L Yield E_rC_{50} (frond number) = 1.2 mg/L NOEC (frond number) = 0.45 mg/L E_rC_{50} (dry weight) = 1.2 mg/L NOEC (dry weight) = 0.45 mg/L (Report SD2006/03296/00)
Daphnia Reproduction test (OECD 211)	Reproduction: EC_{50} (21 day) > 0.12 mg/L LOEC (21 day) = 0.34 mg/L NOEC (21 day) = 0.12 mg/L Immobility: EC_{50} (21 day) > 0.19 mg/L LOEC (21 day) = 0.34 mg/L NOEC (21 day) = 0.12 mg/L (Report 2014N194420_00)
Fish Early Life Stage Toxicity Test (OECD 210)	Hatching: LOEC (28 day) = 0.53 mg/L NOEC (28 day) = 0.16 mg/L Larval Survival: LOEC (28 day) = 0.16 mg/L NOEC (28 day) = 0.052 mg/L Lenght and Weight: LOEC (28 day) = 0.16 mg/L NOEC (28 day) = 0.052 mg/L (Report 2014N194417_00)
Activated Sludge Respiration Inhibition Test (OECD 209)	EC_{50} > 320 mg/L NOEC = 32 mg/L (Report SD2005/01959/00)

Source: (Report SD2006/02328/00), (Report 2014N196411_00), (Report SD2006/00090/00), (Report SD2006/03293/00), (Report 2014N209349_00), (Report SD2006/03296/00), (Report 2014N194420_00), (Report 2014N194417_00), (Report SD2005/01959/00)

The partition coefficient of the test material was determined using the shake-flask method, Method A8 of Commission Directive 92/69/EEC, at nominal pHs of 5, 7 and 9. From structural information and pH dependent water solubility data supplied by the Sponsor; the partition coefficient of the test material will decrease with an increase in aqueous phase pH due to ionisation of the carboxylic acid and then phenol functional groups. The partition coefficient at pH 5 therefore is anticipated to be log10 P_{ow} >4.52. This limit value originates from the experimental value obtained at pH 7, as the degree of ionisation of the test material will be decreased at pH 5, reducing solubility in the aqueous phase and hence increasing the partition coefficient. However the reported experimental limit value was a result of difficulties encountered in recovering the test material from pH 5 aqueous media, such that a higher, definitive partition coefficient value could be determined at pH 7 due to the improved performance of the analytical methods employed.

The inherent ultimate biodegradability of eltrombopag was assessed under standard test conditions. The procedure followed was based upon the OECD guideline 302C Modified MITI Test (II). The primary biodegradation was determined by high performance liquid chromatography (HPLC) analysis. The results from the carbon dioxide evolution data indicated that eltrombopag attained a mean biodegradation of 14% calculated from oxygen consumption values on Day 28. Results from the compound specific analysis showed that there was a loss of parent compound equivalent to 10% primary biodegradation of eltrombopag on Day 28. It can be concluded that eltrombopag is neither readily nor inherently biodegradable in aqueous biodegradation tests.

As discussed in the Type II variation for HCV (eCTD sequence 0037), eltrombopag was initially investigated for determination of activated sludge sorption isotherm according to OPPTS 835.1110 (Report SD2006/00091/00). However, no determination of the isotherm was possible in this study. This was due to the instability of the test material in aqueous solution at a concentration and pH relevant to the fate of the test material in the waste water treatment plant. At the time it was not possible to overcome these technical challenges and, therefore, it was concluded that those physico-chemical characteristics of eltrombopag that contributed to the failure of this study were the same as those which would likely give rise to a Koc value for eltrombopag of greater than 10'000 L/kg ostensibly triggering the need for a Phase II Tier B analysis in soil. However, since this submission it was found that another CRO with better analytical capabilities was able to conduct a soil/sludge adsorption study (OECD 106) with eltrombopag. In this study, the adsorption and desorption of eltrombopag was determined in three soils and two municipal sewage sludges using the batch equilibrium method according to the OECD test guideline no. 106. The following soils/sludges were used: soil I (Speyer 2.3; sandy loam), soil II (Hagenthal; silt loam), soil III (Mechtildshausen; loam), sludge I (Füllinsdorf) and sludge II (Sissach). In order to limit microbial degradation of the test item during the experiment, all soils and both sludge were treated by γ-irradiation before use. Adsorption kinetics were determined for a soil/sludge-to-solution ratio of 1/100 (1 g soil to 100 mL 0.01 M CaCl2) and a test item concentration of 0.027 µg/mL. For all soils and sludge species, the level of adsorption (adsorbed fraction of applied) almost reached equilibrium after 48 hours of agitation due to its high adsorption. The fraction of adsorption after 48 hours was 94.1%, 92.7% and 94.6% of the applied radioactivity for soils I to III, respectively and 95.8% and 95.5% for sludge I and II, respectively. The mass balance, determined for all soils/sludge after 48 hours of adsorption, showed overall mean recoveries of 99.9%, 104.4% and 105.4%

for the soils and 113.3% and 108.8% for both sludges, respectively. The higher recoveries in both sludge species are due to the high amount of non-extractable radioactivity in comparison to the soils and the small amount of material combusted. The mean K_{oc} adsorption value for the soils is 132,556 mL/g and 8,119 mL/g for the sludge. The adsorption showed to be independent of the cation exchange capacity, organic carbon or clay content of the soils. A slight dependence of the adsorption from the pH could be observed. Desorption kinetics were followed 2, 5, 24 and 48 hours after the 48 hours adsorption period. Very little desorption was observed for all soils/sludge throughout the experiment. The percent of the desorbed radioactivity amounted to 3.5%, 2.6%, 2.5%, 0.9% and 0.9% of adsorbed for soils I, II, III and sludge I and II, respectively after 48 hours of desorption. The mean K_{oc} desorption value for the soils was 302,493 mL/g and 30,492 mL/g for the sludge.

The route and rate of degradation of [^{14}C]eltrombopag olamine in two aquatic systems (river and pond) under aerobic conditions were investigated at 20 °C in the dark. [^{14}C]eltrombopag olamine dissipated from the water phase mainly due to rapid degradation and dissipation into the sediment layer. In the water phase eltrombopag olamine immediately degraded to transformation product M4 with the DT_{50} reached immediately after application. The immediate transformation/ degradation of eltrombopag olamine in the water phase is proposed to be driven by abiotic processes, as the dilute biomass associated with 'competent' degraders would be too reduced to cause such an abrupt biotic change. The rapid dissipation of [^{14}C]eltrombopag olamine from the aqueous phase led to a subsequent accumulation in the sediment compartment. Between 10 and 20% degradation was observed after 106 days exposure. The minimal data generated did not allow for the calculation of DT_{50} values with a high degree of statistical confidence but indicated that eltrombopag olamine should be classed as 'Persistent' in sediment when compared to the freshwater sediment trigger levels documented in ECHA guidelines. The percentage of applied radioactivity attributable to M4 in the water compartment of both test systems decreased rapidly, and consequently collected in the 'extractable' compartments of the sediments. Besides eltrombopag olamine and M4, up to eight degradation products were detected. With the exception of M4 no other degradation products exceeded 10% of the applied radioactivity at any time during the study. The formation of radioactive carbon dioxide and other volatile products was insignificant in both aquatic systems. Non-extractable radioactivity increased throughout the course of the study. The majority of non-extractable radioactivity could be attributed to the insoluble humin fraction of the sediment.

While the conclusion that identification of transformation product M4 is not required was accepted within previous marketing authorisation procedures, it is acknowledged that, according to guidance given in OECD TG 308, M4 should be identified. However, as samples from this water/ sediment study have already been discarded, identification of M4 is not feasible anymore.

Eltrombopag was exposed to UV light and measurement of light absorption revealed that this substance does exhibit significant absorption above 290 nm suggesting that degradation via direct photolysis may be a viable environmental depletion mechanism.

In conclusion, eltrombopag is not water soluble but is slightly water soluble at basic pH. It is not likely to partition to air from water very readily. It is neither readily nor inherently biodegradable and is not expected to be extensively mineralized (converted to CO_2). However,

removal of the parent by adsorption to sludge is expected at the STP to a moderate extent. This substance is moderately lipophilic at neutral pH, and the water/soil distribution coefficient (K_{oc}) suggests that in the aquatic environment it is expected to sorb to suspended particles and sediment to a significant extent. Although this substance is considered to be persistent it is expected to be relatively immobile in the aquatic environment.

Phase II-Tier A chronic aquatic toxicity studies have been performed with eltrombopag using duckweed (*Lemna minor*), daphnia, fish and micro-organisms. All concentrations are expressed in terms of eltrombopag free acid.

The impact of eltrombopag on the respiration rate of activated sludge was assessed according to Guideline 209 (Adopted 1984) of the Organisation for Economic Co-operation and Development (OECD). In the definitive test activated sludge was exposed to eltrombopag free acid at concentrations of 1, 3.2, 10, 32, 100 and 320 mg/L. The effect of the test material on the respiration of activated sewage sludge gave a 3-hour EC_{50} greater than 320 mg/L of eltrombopag free acid. The No Observed Effect Concentration (NOEC) after 3 hours exposure was determined to be 32 mg/L.

The effects of eltrombopag on the growth of green algae was determined during a 72-hour acute growth inhibition toxicity test conducted in accordance with OECD Chemicals Testing Guideline No. 201 Alga, Growth Inhibition Test (adopted 23 March 2006). An initial range-finding test was undertaken to assess the effect of the test material on the growth of the unicellular green alga, *Scenedesmus subspicatus*. The test material was known to form a coloured solution and hence a modified algal inhibition test design was employed in order to overcome any effects that the coloured solution may exhibit in terms of inhibition of growth due to light adsorption. The results of the algal range-finding test indicated that the test material significantly adsorbed light at one of the wavelengths required for photosynthesis (460 nm) and that significant inhibition of growth occurred despite the use of the modified test design. Therefore following current regulatory advice it was considered appropriate to change the test method to a Lemna Growth Inhibition Test. The method followed that described in the OECD Guideline "Lemna Growth Inhibition Test (March 2006)", modified by the use of a black, non- reflective surface. For the purpose of the definitive test, the test material was prepared as a saturated solution. Following a preliminary range-finding test, *Lemna minor* was exposed to an aqueous solution of the test material at time weighted mean measured test concentrations of 0.025, 0.13, 0.45, 1.9 and 7.9 mg free acid/l (three replicate flasks per concentration) for a period of 7 days, under constant illumination at a temperature of 24 ± 2°C. The test solutions were renewed on days 2 and 4. The number of fronds in each control and treatment group was recorded on days 0, 2, 4 and 7 along with observations on plant development. In addition the dry weight of the fronds in each control and treatment group was determined on Day 7. Analysis of the saturated solutions on Days 0, 2 and 4 (fresh media) showed measured concentrations of 73 to 118 mg free acid/l. The preliminary chemical analysis from the Acute Toxicity to *Daphnia magna* test indicated that a saturated solution prepared in an identical manner gave a measured concentration of 50 mg free acid/l. The 7-day growth rate (ErC_{50}), and yield (EyC_{50}) toxicity values were determined to be 4.10 and 1.20 mg/L respectively. Under the conditions of this test, eltrombopag was considered to be toxic to *Lemna minor* at nominal concentrations as significant effects on growth relative to the control treatment were observed.

The impact of the test item eltrombopag olamine on the survival and reproduction of *Daphnia magna* was investigated in a semi-static test over 21 days. The test was conducted according to OECD Guidelines for Testing of Chemicals, No. 211 (2012) and the Commission Regulation (EC) No 440/2008, C.20: "*Daphnia mag*na Reproduction Test". The nominal concentrations tested were 0.020, 0.063, 0.20, 0.63 and 2.0 mg/L. Additionally, a control was tested in parallel. The test item concentration in the freshly prepared and aged application solution ranged from 108 to 111% of the nominal value. This shows the correct preparation of the application solution and stability of the test item in the application solution during the renewal period of 7 days. The measured test item concentrations in the freshly prepared test media of the nominal concentrations of 0.063 to 0.63 mg/L were between 66 and 117% of the nominal values at the start of the test medium renewal periods. In the stability control samples without food particles and daphnids, the measured concentrations were between 50 and 91% of the nominal values at the end of the test medium renewal periods of 48 to 72 hours. The following 21-day EC_{50} and NOEC/LOEC values for the reproduction rate and immobility of the daphnids were calculated on the basis of mean measured concentrations of the test item. In conclusion, taking into account the effects of eltrombopag olamine on survival and reproduction of the test animals, the 21-day NOEC was 0.15 mg eltrombopag olamine/L corresponding to 0.12 mg eltrombopag free acid/L. The 21-day LOEC was 0.44 mg eltrombopag olamine/L corresponding to 0.34 mg eltrombopag free acid/L, due to the mortality of *Daphnia magna* at this mean measured concentration.

The toxicity of eltrombopag olamine to zebra fish (*Danio rerio*) was investigated in an early-life stage toxicity test according to the OECD Guideline for Testing of Chemicals, No. 210, "Fish, Early-life Stage Toxicity Test", 1992. Freshly fertilized eggs of zebra fish were exposed to test media containing the test item at nominal concentrations of 0.0080, 0.025, 0.080, 0.25 and 0.80 mg/L under flow-through conditions for the test period of 35 days. Additionally, a control was tested in parallel. At the start of the test, 60 eggs each (divided into 4 replicates) were distributed to the test concentrations and control. The eggs, larvae and juvenile fish were observed for toxic effects on their development, growth and survival. The test item concentration in the freshly prepared and aged application solution ranged from 109 to 111% of the nominal value. This shows the correct preparation of the application solution and stability of the test item in the application solution during the renewal period of 8 days. The test item concentrations in the analyzed test media of nominal 0.025 to 0.80 mg/L varied in the range from 55 to 121% of the nominal values. The mean measured test item concentrations (calculated as the arithmetic mean of all measurements of each test concentration) were expressed in terms of eltrombopag olamine and eltrombopag free acid (77.8% in test item). The overall NOEC of eltrombopag olamine for early life stages of zebra fish was determined to be the mean measured concentration of 0.067 mg eltrombopag olamine/L (corresponding to 0.052 mg eltrombopag free acid/L), since no toxic effect on the eggs, larvae or fish was observed up to and including this concentration. The overall LOEC was determined to be the mean measured concentration of 0.20 mg eltrombopag olamine/L (corresponding to 0.16 mg eltrombopag free acid/L), due to the statistically significantly reduced survival at that concentration.

In conclusion, eltrombopag is not toxic to activated sludge microbial populations. In chronic studies this substance exhibits toxicity to duckweed, dapnia and fish. However, it should be

noted that surface water concentrations are unlikely to reach a level where effects are expected to be observed.

4.1.2 Calculation of PNECs using assessment factors

The $PNEC_{surface\ water}$ derived from the NOEC for length and weight of zebrafish as the most sensitive species is therefore 52.0 µg/L / 10 = 5.2 µg/L.

For microorganisms, an assessment factor of 10 is used, hence the $PNEC_{microorg.}$ derived from the activated sludge respiration inhibition study is 32'000 µg/L / 10 = 3'200 µg/L.

The $PNEC_{groundwater}$ is based on the NOEC of the test with *Daphnia sp.* and applying an assessment factor of 10 thus calculates as 120 µg/L / 10 = 12 µg/L.

4.1.3 Refinement of $PEC_{surface\ water}$

The Tier A PEC is based on the "total residue approach", neglecting depletion by biotic and abiotic processes. The Phase II Tier A $PEC_{surface\ water}$ has been calculated using epidemiological data for all currently registered and newly applied for indications, i.e. cHCVaT, SAA, cITP and first line SAA (total PEC) based on the assumption that 100% of drug substance used enters the sewage treatment plant unchanged and passes through into the aquatic environment.

Idiopathic thrombocytopenia purpura (ITP) is an autoimmune disorder characterized by autoantibody-induced platelet destruction and reduced platelet production, leading to a chronically low peripheral blood platelet count (<150,000/µL). As indicated by the term 'idiopathic', the exact etiology of ITP is unknown. Anti-platelet antibodies are thought to play a role in mediating destruction of platelets, and may impede the generation of platelets in the bone marrow.

The prevalence of ITP in the population is known to be very low. As laid out in the latest EU Safety Risk Management Plan for eltrombopag the prevalence of ITP in the EU is estimated to be 2.1 in 100'000 inhabitants.

Chronic hepatitis C (cHCV) infection is a serious disease, especially for those with significant thrombocytopenia (<100 Gi/L). In this population the annualized incidence rate for hepatocellular carcinoma or clinical hepatic decompensation (ascites, hepatic encephalopathy, variceal bleeding, spontaneous bacterial peritonitis) is approximately 8%. Every year more than 7% die or undergo liver transplantation. Over a 5-year period 1 out of 4 such patients will die.

The aetiology of thrombocytopenia in HCV-infected patients is multi-fold and severity correlates with the severity of liver disease. Approximately 20% of HCV-infected individuals develop liver cirrhosis, with thrombocytopenia as a clinical marker of progression to more severe hepatic impairment. Consequently, low platelet counts are predictive of poorer outcomes. Thrombocytopenia is also caused, or further aggravated, by interferon-based antiviral therapy due to its myelosuppressive effects. Eltrombopag, working as a supporting care agent to increase the platelet count prior to and throughout interferon-based treatment of HCV infection initiate and help optimise and maintain the dose and duration of their antiviral therapy, improving the likelihood of achieving a sustained virologic response (SVR).

In Europe, the HCV prevalence estimates show high variability between countries ranging from ≤ 0.5% in the northern European countries to ≥ 3% in the Romania and rural areas in Greece, Italy and Russia (according to EU Safety Risk Management Plan). The prevalence of thrombocytopenia in patients with HCV infection is estimated to be 5.8 %. Based on an EU population of 512.596.403 in January 2018, this results in 891'918 patients with cHCV associated thrombocytopenia or a prevalence of 174 in 100'000 for the EU.

Severe aplastic anaemia (SAA) is a rare, life-threatening, acquired bone marrow failure disease characterized by tri-lineage marrow hypoplasia and a lack of haematopoietic stem and progenitor cells (HSPC) due to an immune-mediated attack on the bone marrow. There is no standard of care for SAA patients with an insufficient response to immunosuppressive therapy who do not have suitable donor for bone marrow transplantation.

For SAA no data on prevalence is available, however, information on incidence with a maximum of 2.54 cases in 1 million people found within the EU countries for which information is available, shows that SAA is a very rare disease (EU Safety Risk Management Plan). In the absence of prevalence data, the market penetration factor for SAA is calculated based on the maximum incidence for this disease for the EU.

PECsurface water = (DOSEai * Fpen) / (WASTEWinhab * DILUTION)

= (75 mg/inhabitant/day * 0.000021) / (200 L/inhabitant/day * 10)

= 0.0007875 µg/L for ITP

PECsurface water = (DOSEai * Fpen) / (WASTEWinhab * DILUTION)

= (100 mg/inhabitant/day * 0.00174) / (200 L/inhabitant/day * 10)

= 0.087 µg/L for cHCV associated thrombocytopenia

PECsurface water = (DOSEai * Fpen) / (WASTEWinhab * DILUTION)

= (150 mg/inhabitant/day * 0.00000254) / (200 L/inhabitant/day * 10)

= 0.00019 µg/L for SAA

Where:

DOSEai = 75 mg/patient/day for ITP;
= 100 mg/patient/day for cHCV associated thrombocytopenia
= 150 mg/patient/day for 6 months for SAA

Fpen = 2.1 in 100'000 inhabitants; 0.0021 % for ITP
= 174 in 100'000 inhabitants; 0.174 % for cHCV associated thrombocytopenia
= 0.254 in 100'000 inhabitants; 0.000254 % for SAA

WASTEWinhab = 200 L/inhabitant/day

DILUTION = 10

Overall $PEC_{surface\ water}$ = 0.0007875 µg/L + 0.087 µg/L + 0.00019 µg/L = 0.088 µg/L

4.1.4 Calculation of $PEC_{microorg}$

Based on the default dilution factor of 10 used between surface water and sewage treatment plants, the $PEC_{microorg}$ is ten times higher than the $PEC_{surface\ water}$.

$PEC_{microorg}$ = 0.88 µg/L

4.1.5 Calculation of $PEC_{groundwater}$

According to the guideline, the $PEC_{groundwater}$ can be assumed to be typically 0.25 times the $PEC_{surface\ water}$. This leads to a $PEC_{groundwater}$ of 0.022 for eltrombopag free acid.

4.2 Outcome of Tier A fate and effects analysis

4.2.1 Surface water assessment

Refined $PEC_{surface\ water}$ = 0.088 µg/L

$PNEC_{surface\ water}$ = 5.2 µg/L

$PEC/PNEC_{surface\ water}$ = 0.088 µg/L / 5.2 µg/L = 0.017

4.2.2 Microorganisms / sewage treatment plant assessment

$PEC_{microorg.}$ = 0.88 µg/L

$PNEC_{microorg}$ = 3'200 µg/L

$PEC/PNEC_{microorg.}$ = 0.88 µg/L / 3'200 µg/L = 0.000275

4.2.3 Groundwater assessment

$PEC_{groundwater.}$ = 0.022 µg/L

$PNEC_{groundwater}$ = 12.0 µg/L

$PEC/PNEC_{groundwater}$ = 0.022 µg/L / 12.0 µg/L = 0.0018

Table 4-2 Hazard/risk assessment eltrombopag olamine

Hazard/risk criterion	Data requirement
n-octanol/water coefficient (log Kow) > 3	Fish bioconcentration study conducted in Tier B
Adsorption – Desorption: sludge Koc < 10'000	Sorption to sludge below the trigger value for a Tier B terrestrial assessment. No terrestrial assessment required.
$PEC_{surface\ water}$ / $PNEC_{surface\ water}$ < 1	0.088 µg/L / 5.2 µg/L = 0.017
$PEC_{microorg}$ / $PNEC_{microorg}$ < 0.1	0.88 µg/L / 3'200 µg/L = 0.000275
$PEC_{groundwater}$ / $PNEC_{groundwater}$ < 1	0.022 µg/L / 12.0 µg/L = 0.0018

4.3 Conclusion Tier A risk assessment

The outcome of the Phase II-Tier A risk assessment indicates no noteworthy risk potential for surface waters, groundwater and microoganisms in sewage treatment plants, with the highest risk ratio of 0.017 found for surface waters.

The sludge sorption coefficient for eltrombopag, Koc = 8'119, is less than 10'000 indicating that this substance will not bind extensively to sludge and is unlikely to reach the terrestrial compartment, through the application of sewage sludge, to a significant extent. Accordingly, an environmental risk assessment for this substance in the terrestrial compartment is not required in Tier B.

Results from the water sediment study (OECD 308) demonstrate significant shifting of eltrombopag to the sediment. The criterion for sediment studies is met if more than 10% of the substance at any time point at or after 14 days is present in sediment. Accordingly, a study to determine effects in a sediment dwelling organism is warranted.

Eltrombopag has a moderately high partition coefficient (log Dow = 4.52 at pH 7) suggesting it has the potential to bioaccumulate in the tissues of aquatic organisms. As this substance adsorbs to sediment and is also persistent in the environment, a bioaccumulation study is warranted in Tier B.

5 Phase II – Tier B: Extended environmental fate and effects analysis

5.1 $PEC_{surface\ water}$ refinement and PEC_{sludge} calculation

In Tier B the $PEC_{surface\ water}$ may be refined with information from STP modelling using the SimpleTreat model by incorporating adsorption of substances to sewage sludge in STPs, using the data from the estimation of the adsorption coefficient in sewage sludge from two sewage treatment plants.

Applying SimpleTreat STP modelling and an $Elocal_{water}$ of 2136.75 mg/day (see calculation presented below) and using REACH Guidance on information requirements and chemical safety assessment Chapter R16.: Environmental Exposure Estimation (European Chemicals Agency, ECHA, October 2012) suggests 53 % elimination of eltrombopag from waste water (35.03 % via primary sludge and 17.79 % via surplus sludge) and 47.18 % emission of eltrombopag to effluent (Eltrombopag SimpleTreat.pdf).

$Elocal_{water}$ = DOSEai * $F_{excreta}$ * F_{pen} * CAPACITYstp

ITP: 75 mg/inhabitant/day * 1 * 0.000021 * 10'000 inhabitants = 15.75 mg/day

cHCVaT: 100 mg/inhabitant/day * 1 * 0.00174 * 10'000 inhabitants = 1740 mg/day

SAA: 150 mg/inhabitant/day * 1 * 0.000254 * 10'000 = 381 mg/day

Sum $Elocal_{water}$ = 15.75 + 1740 + 381 = 2136.75 mg/day

With:

DOSEai = 75 mg/patient/day for ITP

= 100 mg/patient/day for cHCV associated thrombocytopenia

= 150 mg/patient/day for 6 months for SAA

CAPACITYstp = 10'000 inhabitants (R.16 Table R.16-10)

$F_{excreta}$ = 100 %

F_{pen} = 2.1 in 100'000 inhabitants; 0.0021 % for ITP

= 174 in 100'000 inhabitants; 0.174 % for cHCV associated thrombocytopenia

= 0.254 in 100'000 inhabitants; 0.000254 % for SAA

Applying the outcome of the SimpleTreat calculations the local surface water concentration can be refined as:

$PEC_{SURFACEWATER}$ = ($Elocal_{water}$* $F_{stp\ water}$) / ($WASTEW_{inhab}$ * $CAPACITY_{stp}$ * FACTOR * DILUTION)

= (2130.75 mg/day * 0.4718) / (200 L/inhabitant/day * 10'000 inhabitants * 10) = 0.050 µg/L

With:

$Elocal_{water}$ = 2130.75 mg/day

$Fstp_{water}$ = 47.18 %

$WASTEW_{inhab}$ = 200 L/inhabitant/day (R.16 Table R.16-10)

CAPACITYstp = 10'000 inhabitants (R.16 Table R.16-10)

FACTOR: Kp_{susp} = Foc_{susp} * Koc = 0.1 * 8'188.5 L/kg = 811.85 L/kg (R.16 Equation 16-6)

Foc_{susp}: 0.1 kg_{oc}*kg_{solid}^{-1} (R.16 Table R.16-9)

Koc = (8'534 L/kg + 7'703 L/kg) / 2 = 8'188.5 L/kg (average from Koc from two sludges, see Table 4.1)

Dilution = 10

While the risk ratio calculated for surface waters in Tier A does not indicate a risk for this environmental compartment and therefore no refinement is required, it can be noted that applying the refined Tier B $PEC_{surface\ water}$ for the calculation of the risk ratio results in a $PEC/PNEC_{surface\ water}$ of 0.0096.

Subsequently, the refined Tier B $PEC_{surface\ water}$ is used to calculate $PEC_{sediment}$.

5.2 Extended effects analysis

5.2.1 Water sediment effects

The water-sediment study has shown that the level of eltrombopag in sediment at 14 days is greater than 10% thereby exceeding the guidance criterion for sediment studies. The toxicity

of eltrombopag was evaluated on the sediment-dwelling non-biting midge *Chironomus riparius* by exposure in a static sediment-water test system over a 28-day period in accordance with the requirements of OECD Chemicals Testing Guideline No. 218 Sediment-Water Chironomid Toxicity Test Using Spiked Sediment.

Following a preliminary range-finding test, larvae of *Chironomus riparius* were exposed in groups of 80 (four replicates of 20 larvae per concentration) to formulated sediment spiked with test item over a range of concentrations of 100, 180, 320, 560 and 1000 mg/Kg for a period of 28 days. The numbers of emerged adult midges were recorded daily.

Examination of the numbers of male and female adult midges emerged) showed no biological significance between the numbers of male and females. Therefore, it was considered that the test item had no significant effect on the sex ratio of emerged adults.

The toxicity of the test item to the sediment-dwelling larvae of *Chironomus riparius* has been investigated and based on nominal concentrations gave 28-Day EC_{15} and EC_{50} (reduction in emergence) of 350 and 690 mg/Kg respectively. The No Observed Effect Concentration was 560 mg/Kg and the Lowest Observed Effect Concentration was 1000 mg/kg. The results based on geometric mean measured concentrations gave 28- Day EC_{15} and EC_{50} (reduction in emergence) of 66 and 130 mg/Kg respectively. The No Observed Effect Concentration was 104 mg/Kg and the Lowest Observed Effect Concentration was 185 mg/Kg. The 28-Day EC_{15} and EC_{50} values for development rate were not determined as only -0.4% reduction in development rate was determined for the 560 mg/Kg test group (the highest concentration where development rate could be calculated). The No Observed Effect Concentration was determined to be 560 mg/Kg (based on nominal concentration and 104 mg/kg based on estimated geometric mean measured concentrations).

Table 5-1 Tier B toxicity data for eltrombopag olamine

Development of sediment-dwelling organisms (OECD 218)	EC50 (emergence) = 130 mg/kg LOEC (emergence) = 185 mg/kg NOEC (emergence) = 104 mg/kg NOEC (development) = 104 mg/kg

Source: (Report 2016N274772_0)

The $PEC_{sediment}$ can be calculated following REACH Guidance on information requirements and chemical safety assessment Chapter R16.: Environmental Exposure Estimation (European Chemicals Agency, ECHA, October 2012):

$PEC_{local\ sed} = (K_{susp\text{-}water} / RHO_{susp}) * PEC_{local\ water} * 1000 = (3314.8\ L/m^3 / 1150\ kg/m^3) * 0.05\ \mu g/L * 1000 = 144.12\ \mu g/kg$ (Equation R.16-35)

With:

$RHO_{susp} = 1'150\ kg/m^3$ (Equation R. 16-16)

$K_{susp\text{-}water} = Fwater_{susp} + Fsolid_{susp} * (Kp_{susp} / 1000) * RHO_{solid} =$

$0.9\ m_{water}^3/m_{susp}^3 + 0.1\ m_{solid}^3/m_{susp}^3 * (13'255.6\ L/kg_{solid} / 1000) * 2500\ kg/m^3 = 3314.8\ L/m^3$

(Equation R.16-7)

With:

RHO_{solid} = 2'500 kg/m^3 (Table R. 16.9)

Kp_{susp} = Foc_{susp} * Koc_{soil} = 0.1 kg_{oc}/kg_{solid} * 132'556 L/kg = 13'255.6 L/kg_{solid} (Equation R.16.6)

Koc_{soil} = 132'556 ml/g (Average from three soils, i.e. 155'386, 97'529 and 144'754 mL/g)

Foc_{susp} = 0.1 kg_{oc}/kg_{solid} (Table 16.9)

In order to account for the fact that the effect concentration of the sediment toxicity study is expressed as a dry weight concentration, the $PEC_{sediment}$ calculated above, which relates to wet sediment is multiplied with a conversion factor of 4.6, resulting in a dry weight $PEC_{sediment}$ of 662.96 µg/kg.

The $PNEC_{sediment}$, based on the NOEC for the development of the sediment-dwelling larvae of *Chironomus riparius* (Table 5-1) including an assessment factor of 100, is 1'040 µg/L.

The risk ratio for sediment therefore calculates as:

$PEC/PNEC_{sediment}$ = 662.96 µg/kg / 1'040 µg/kg = **0.64**

This result indicates that eltrombopag does not constitute a risk to sediment compartments.

5.3 Bioaccumulation

Table 5-2 Tier B bioaccumulation data

Bioconcentration in fish (OECD 305)	**Low concentration: 0.0025 mg/L** BCFss = 14 L/kg BCFk = 29 L/kg BCFss lipid-normalised = 336 L/kg **High concentration: 0.025 mg/L** BCFss = 14 L/kg BCFk = 16 L/kg BCFss lipid-normalised = 130 L/kg

Source: (Report 2011N113982_01)

The measured octanol-water partition coefficient (log Dow @pH 7 = 4.52) of eltrombopag is above 3.0 indicating that eltrombopag could have a tendency to sorb to lipid surfaces and therefore bioaccumulate in the tissues of aquatic organisms.

A study was performed to assess the bioconcentration potential of the test item in rainbow trout (*Oncorhynchus mykiss*). The method followed was that described in the OECD Guideline for the Testing of Chemicals (1996) No 305 "Bioconcentration: Flow-Through Fish Test.

Rainbow trout were exposed, in groups of 52, to an aqueous solution of the test item at concentrations of 0.0025 and 0.025 mg/l for a period of 14 days under dynamic test conditions. Samples of test fish were taken from the solvent control, 0.0025 and 0.025 mg/l test groups on days 5, 7, 10, 12 and 14 and the concentration of test item in the fish tissues determined. After 14 days exposure the remaining fish were subjected to a depuration period of 10 days.

Samples of test fish were taken from the solvent control and 0.0025 and 0.025 mg/l test groups on days 3, 5, 7 and 10 of the depuration phase and the concentration of test item determined. The Bioconcentration Factors at steady state (BCF_{ss}) for the test item based on total radioactivity in the whole fish after 14 days were calculated to be 14 at concentrations of 0.0025 and 0.025 mg/l (equivalent to 0.0020 and 0.020 mg/l as free base respectively). The Kinetic Bioconcentration Factors (BCF_k) were also calculated and were shown to be 29 at a concentration of 0.0025 mg/l and 16 at a concentration of 0.025 mg/l after 14 days.

The uptake and depuration rate constants, k1 and k2, were determined to be 4.4148 and 0.1515 for the 0.0025 mg/l test concentration and 3.0805 and 0.1957 for the 0.025 mg/l test concentration. As the test item had a log Kow of 4.52, following the recommendations of the test guidelines, the BCF values were also calculated as a function of the lipid content. These BCF values were higher than those determined in the whole fish results thereby suggesting that the accumulation of the test item was into the lipid content of the fish. However, as the values were below 2000, the test item would not be classified as bioaccumulative according to the PBT assessment criteria. The Bioconcentration Factors at steady state (BCF_{ss}) for the test item as a function of lipid content after 14 days was calculated to be 336 and 130 at concentrations of 0.0025 and 0.025 mg/l (equivalent to 0.0020 and 0.020 mg/l as free base), respectively. The Kinetic Bioconcentration Factors (BCF_k) were also calculated and were shown to be 280 at a concentration of 0.0025 mg/l and 136 at a concentration of 0.025 mg/l after 14 days.

The BCFss has been recalculated taking into account three successive analysis of Cf and normalisation to 5% lipid content as follows:

Low concentration (0.0025 mg/L): BCFss as function of the lipid content (taken from study Report 2011N113982_01, table 3): mean value from day 7 – 12 (325, 330, 352) = 336

High concentration (0.025 mg/L): BCFss as function of the lipid content (taken from study Report 2011N113982_01, table 3): mean value from day 7 – 14 (137, 144, 105, 135) = 130

Eltrombopag olamine (SB-497115-GR) was shown not to significantly accumulate in fish tissue. At the end of the 10-Day depuration period 88% and 85% of eltrombopag olamine (SB-497115-GR) was eliminated from the fish tissues for the 0.0025 and 0.025 mg/l test concentrations respectively. Although this was less than the 90% elimination detailed in the guideline, it was considered that the results of the test were valid given the low BCF values obtained during the study. Based on this information the biological half-life of the test item is considered to be between 3 and 10 days.

Based on the lipid normalised BCFss values of 336 and 130 for the low dose and high dose exposure, respectively, remaining below the criteria for a bioaccumulative substance of a BCF of 2000, eltrombopag is not considered a bioaccumulative substance.

6 Overall conclusion

According to current guidance, the fate of only one component of the eltrombopag tablets (12.5, 25, 50, 75 and 100 mg) and eltrombopag PfOS (25 mg) product, including packaging, requires consideration. This component is the drug substance eltrombopag.

On the basis of the original Phase I assessment of eltrombopag which was informed by the registration of eltrombopag tablets for the Orphan Indication ("for the treatment of previously-treated patients with chronic idiopathic thrombocytopenic purpura (ITP) to increase platelet counts and reduce or prevent bleeding"), no Phase II environmental fate and effect analysis was required for inclusion in the original environmental assessment. However, some environmental fate and effect data were available for that submission and these data were presented in the original application for completeness.

Subsequently, using the Guidance on "Environmental Risk Assessment of Medicinal Products for Human Use" (EMEA/CHMP/SWP/4447/00 corr 2) a Phase I calculation for prediction of PEC has been made and the calculated PEC for eltrombopag (0.75 µg/L) was above the trigger value of 0.01 µg/L. Thus, a full Phase II-Tier A assessment has been carried out and extended fate and effects analysis has been conducted as part of Tier B.

Following administration to man, unchanged eltrombopag will be excreted in urine and faeces. Excreted eltrombopag will enter the sewage treatment plant where the physical and chemical characteristics of this substance indicate that it will be partitioning to the aqueous phase to a significant extent. Eltrombopag is not predicted to distribute to the terrestrial compartment to a significant extent. This substance is not readily nor inherently biodegradable and is not expected to be extensively mineralized (converted to CO2). Eltrombopag is moderately lipophilic at neutral pH, and the water/soil distribution coefficient (K_{oc}) suggests that in the aquatic environment it is expected to sorb to suspended particles and sediment to a significant extent.

In accordance with the EMA ERA guideline the PEC for surface water has been refined based on epidemiological data, i.e. prevalence of the diseases for all indications (HCV, SAA, pITP and first line SAA). Applying the more realistic market penetration factors based on disease prevalence resulted in a $PEC_{surface\ water}$ value of 0.088 µg/L. This is a conservative value in that it has been assumed that 100% of the patients with the respective disease will be treated with elrombopag. Moreover, it is assumed that, after patients' usage, 100% of the drug substance enters the aquatic environment. Metabolism of the active drug and fate of the compound at the STP have not been taken into account.

Eltrombopag has a full Tier A of chronic toxicity testing. This substance is not toxic to activated sludge microbial populations. In chronic studies this substance exhibits toxicity to duckweed (*Lemna minor*), dapnia and fish. However, it should be noted that surface water concentrations are unlikely to reach a level where effects are expected to be observed. Similarly, eltrombopag is not toxic to the benthic organism *Chironomus riparius* and significant effects are only observed at test concentration > 100 mg/Kg.

According to the guideline a drug substance with a partition coefficient greater than 4.5 should be screened for persistence, bioaccumulation and toxicity in accordance with the ECHA REACH guidelines. Eltrombopag does not fulfil the criteria for bioaccumulation potential and chronic toxicity and therefore it can be concluded that eltrombopag does not fulfil the criteria to be considered a PBT/vPvB (i.e. Persistent/Bioaccumulative/Toxic or very Persistent/very Bioaccumulative) substance as set out in the guidance.

In summation, eltrombopag is a moderately lipophilic water insoluble compound that will significantly partition to the aquatic environment. The terrestrial environment is not expected

to be affected to a significant extent. It is not toxic to activated sludge but has exhibited sub-lethal toxicity to Lemna, Daphnia and fish in chronic studies. Surface water concentrations are unlikely to reach a level where effects are expected to be observed i.e. PEC/PNEC < 1. It can be concluded that this drug substance is unlikely to represent a risk to the aquatic environment.

Other compounds potentially emitted in the use of eltrombopag medical products, i.e., the excipients, and waste packaging materials are introduced into the environment from a wide variety of sources. The amounts of these compounds expected to enter the environment as a result of the use of these products are negligible by comparison.

In spite of the fact that no immediate risk was found for eltrombopag and excretion of parent substance by patients is expected to be low, intake of active pharmaceutical ingredients into surface waters should be avoided as far as possible. Therefore, as with all non-readily biodegradable human medicines, patients should be advised not to dispose of unused eltrombopag via domestic sewage.

Table 6-1 Summary of main study results

Substance (INN/Invented Name): Eltrombopag olamine			
CAS-number: 496775-62-3			
PBT screening		Result	Conclusion
Bioaccumulation potential- log Kow	92/69/EEC Method A8 (shake-flask method)	log Dow > 4.1 at pH 5 log Dow = 4.52 at pH 7 log Dow = 0.96 at pH 9	Potential PBT (Y)
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	BCF (steady-state lipid-normalised) (OECD 305)	336 (low concentration) and 130 (high concentration)	not B
Persistence	DT50 (total water and sediment) (OECD 308)	DT at 12°C: 653, 3244 days (sediment) 1043, 3116 days (total system)	vP
Toxicity	NOEC Fish, Early Life Stage Toxicity (OECD 210)	NOEC = 52 µg/L	not T
PBT-statement :	The compound is not considered as PBT nor vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC surfacewater , default or refined (e.g. prevalence, literature)	1.625	µg/L	> 0.01 threshold

Other concerns (e.g. chemical class)			No
Phase II Physical-chemical properties and fate			
Study type	**Test protocol**	**Results**	**Remarks**
Adsorption-Desorption	OECD 106	K_{oc} sludge = 8'534 and 7'703 mL/g	
		K_{oc} soil = 155'386, 97'529 and 144'754 mL/g K_d sludge = 2'355 and 2'148 mL/g K_d soil = 1'538, 1'307 and 1'723 mL/g	
Ready Biodegradability Test	OECD 302 C	Not inherently biodegradable	
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	DT50 (total water and sediment) > 120 days % shifting to sediment = 36.2 -55.4% of applied radioactivity as parent drug substance (between 7 d to 106 d)	

Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**value**	**Unit**	**Remarks**
Lemna, Growth Inhibition Test	OECD 221	NOEC	450	µg/L	*Lemna minor*
Daphnia sp. Reproduction Test	OECD 211	NOEC	120	µg/L	
Fish, Early Life Stage Toxicity Test	OECD 210	NOEC	52	µg/L	*Danio rerio*
Activated Sludge, Respiration Inhibition Test	OECD 209	NOEC	32'000	µg/L	
Phase IIb Studies					
Bioaccumulation	OECD 305	BCFss (lipid-normalised)	336 (low concentration) and 130 (high concentration)	L/kg	% lipids: 10% at test start; 8% at end of the test
Sediment dwelling organism	OECD 218	NOEC	104	mg/kg	*Chironomus riparius*

7 References

[Eltrombopag SimpleTreat.pdf] SimpleTreat 4.0 Export file, Calculation mode: SimpleTreat 4.0. Version 4.0.8, calculated on August 02, 2018.

NB: The following references and company reports were submitted as a part of the original application for Revolade® and thus copies have not been appended to this application. Copies of all or any of these references can be provided upon request.

EMEA/CHMP/SWP/4447/00 corr 2. Guideline on the Environmental Assessment of Medicinal Products for Human Use. EMEA, June 2006.

REACH Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT Assessment. ECHA, May 2008.

[Report SD2006/02328/00] SB-497115-GR: Determination of Partition Coefficient (Project Number 1127/1302). Final report: 12 October 2006.

[Report SD2006/00091/00] SB-497115-GR: Determination of Activated Sludge Sorption. Isotherm (Project Number 1127/899). Final report: 25 January 2006.

[Report 2014N196411_00] [^{14}C]Eltrombopag Olamine: Adsorption/Desorption Using a Batch Equilibrium Method (OECD 106) (Project Number D80318). Final report: 04 July 2014.

[Report SD2006/00090/00] SB-497115-GR: Assessment of Inherent Biodegradability; Modified MITI (II) test (Project Number 1127/897). Final report: 30 January 2006.

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[Report 2014N209349_00] [^{14}C]Eltrombopag Olamine: Route and Rate of Degradation in Aerobic Aquatic Sediment Systems (OECD 308) (Project Number D80320). Final report: 28 July 2014.

[Report SD2006/03296/00] SB-497115-GR: *Lemna* Growth Inhibition Test (Project Number 1127/1200). Final report: 10 May 2007.

[Report 2014N194420_00] Eltrombopag Olamine: Effect on Survival and Reproduction of Daphnia magna in a Semi-Static Test over Three Weeks (OECD 211) (Project Number D80342). Final report: 13 March 2014.

[Report 2014N194417_00] Eltrombopag Olamine: Toxic Effects to Zebra Fish (Danio rerio) in an Early-Life Stage Toxicity Test (OECD 210) (Project Number D80331). Final report: 24 March 2014.

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8 CV of the author

See Module [1.6.1.1] Information about the expert - Environment