Environmental Risk Assessment of Ivermectin: A Case Study

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ABSTRACT

The veterinary parasiticide ivermectin was selected as a case study compound within the project ERAPharm (Environmental Risk Assessment of Pharmaceuticals). Based on experimental data generated within ERAPharm and additional literature data, an environmental risk assessment (ERA) was performed mainly according to international and European guidelines. For the environmental compartments surface water, sediment, and dung, a risk was indicated at all levels of the tiered assessment approach. Only for soil was no risk indicated after the lower tier assessment. However, the use of effects data from additional 2-species and multispecies studies resulted in a risk indication for collembolans. Although previously performed ERAs for ivermectin revealed no concern for the aquatic compartment, and transient effects on dung-insect populations were not considered as relevant, the present ERA clearly demonstrates unacceptable risks for all investigated environmental compartments and hence suggests the necessity of reassessing ivermectin-containing products. Based on this case study, several gaps in the existing guidelines for ERA of pharmaceuticals were shown and improvements have been suggested. The action limit at the start of the ERA, for example, is not protective for substances such as ivermectin when used on intensively reared animals. Furthermore, initial predicted environmental concentrations (PECs) of ivermectin in soil were estimated to be lower than refined PECs, indicating that the currently used tiered approach for exposure assessment is not appropriate for substances with potential for accumulation in soil. In addition, guidance is lacking for the assessment of effects at higher tiers of the ERA, e.g., for field studies or a tiered effects assessment in the dung compartment. Integr Environ Assess Manag 2010;6:567–587. © 2010 SETAC

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INTRODUCTION

The potential risk of veterinary medicinal products (VMPs) for the environment raised concern much earlier than that of human medical products (HMPs). For example, the impact of parasiticides on the survival of dung beetles was studied more than 30 y ago (Blume et al. 1976). VMPs often reach soils more directly than HMPs, because VMPs such as endo- and ectoparasiticides are regularly applied to pasture animals and intensively reared livestock. Residues can reach soils through 3 main exposure routes: directly via feces, indirectly via spread manure or through wash-off from topically applied products (Halling-Sørensen et al. 1998). VMPs often act as biocides; i.e., they specifically act on target organisms such as bacteria or invertebrates (Boxall et al. 2004). In this respect they are very similar to pesticides and biocidal products. There are even examples in which the same active substance is used for both purposes, i.e., as pesticide and VMP (e.g., deltamethrin). Therefore, similar environmental problems are likely to occur for VMPs as for pesticides. Target (e.g., blow flies or ascaricid roundworms) and nontarget organisms (e.g., dung flies or saprophagous nematodes) can belong to the same taxonomic groups, dipterans and nematodes, respectively. Hence, the respective substances are likely to affect not only target but also nontarget organisms. The main difference between pesticides and VMPs is that the latter are often excreted as a mixture of metabolites and parent compound, whereas pesticides are released directly to the environment as parent compound (Halling-Sørensen et al. 1998).

Avermectins are an important group of VMPs in terms of both their widespread use and their potential environmental risks (Campbell et al. 1983; Strong and Brown 1987). They have been used in agriculture and horticulture for the

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protection of fruits, cotton, vegetables, and ornamentals (Dybas 1989), because they are effective against a wide range of nematodes, mites, and insects (Strong and Brown 1987; Õmura 2008). Avermectins are also used for treatment of river blindness, i.e., onchocerciasis, in humans (Lindley 1987). However, the most extensive use of avermectins is in the control of livestock parasites. The main route of excretion is via feces (Chui et al. 1990), which provides a microhabitat and breeding ground for a very large number of invertebrate species, on which avermectins are known to have deleterious effects.

Avermectins are macrocyclic lactones isolated from the soil actinomycete *Streptomyces avermitilis*. The most well studied avermectin is ivermectin (consisting of ≥80% 22,23-dihydroavermectin B_{1a} and ≤20% 22,23-dihydroavermectin B_{1b}; Figure 1), a synthetic derivative of the naturally occurring avermectin B_1. Ivermectin binds selectively and with high affinity to the ligand glutamate on the ligand-gated chloride ion channels that occur in invertebrate nerve and muscle cells, causing irreversible opening of these channels (Rohrer and Arena 1995; Õmura 2002). Furthermore, ivermectin affects γ-aminobutyric acid (GABA)-related chloride ion channels occurring in the peripheral nervous system of invertebrates and in the central nervous system of vertebrates (Duce and Scott 1985). From a food safety perspective, the margin of safety for ivermectin is attributable to the facts that 1) mammals do not have glutamate-gated chloride channels, and 2) the macrocyclic lactones have a low affinity for other mammalian ligand-gated chloride channels and do not readily cross the blood–brain barrier (Boelsterli 2003; Õmura 2008).

With over 5 billion doses sold worldwide since its market introduction in the early 1980s, ivermectin has become the most widely used antiparasitic drug (Shoop and Soll 2002). It is used regularly as a parasiticide for cattle, pigs, sheep, horses, and dogs (Campbell et al. 1983; Forbes 1993). Oral applications tend to result in sharp excretion peaks, with most of the dose excreted over a few days. Peak elimination of injectable or topical formulations usually occurs within 2 to 7 d posttreatment, followed by a long tail that may sustain for more than 4 to 6 weeks, whereas peak elimination levels of sustained-release formulations may occur over several weeks posttreatment (Floate et al. 2005).

Because of its very high acute toxicity to invertebrates (see, e.g., Blume et al. 1976; Campbell et al. 1983), an environmental risk assessment (ERA) for ivermectin was performed as early as 1986 (USFDA 1986). Several studies have addressed exposure and effects of ivermectin in the environment (e.g., Edwards et al. 2001; Boxall et al. 2004; Floate et al. 2005; Kolar and Kožuh Eržen 2006), but few were carried out according to standardized guidelines. Because of its potential environmental effects and its economic importance, ivermectin was chosen as a case study compound within the EU-funded project ERAPharm.

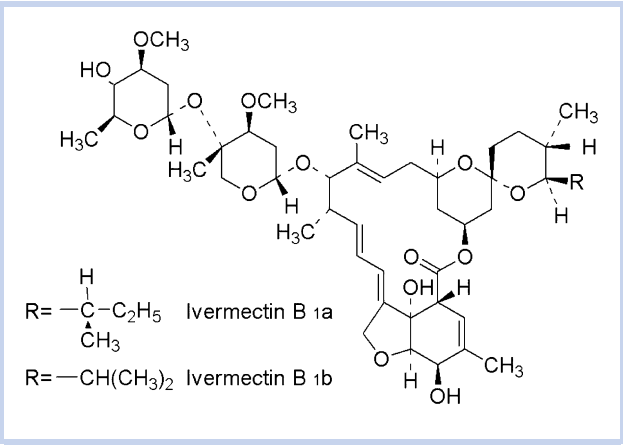

Figure 1. Chemical structure of ivermectin.

In the European Union (EU), the evaluation of the environmental risk of veterinary medicinal products within marketing authorization procedures has been discussed since the mid-1990s (Koschorreck and Apel 2006), and a first guidance document on how to perform the ERA was prepared by the European Medicines Agency in 1997 (EMEA 1997). From this document, the EU, the United States, and Japan harmonized the ERA procedures and prepared 2 guidelines, of which the first focuses on exposure assessment (phase I; VICH 2000) and the second on a tiered risk assessment (phase II; VICH 2004). For the EU, additional guidance in support of the VICH guidelines is provided by EMEA (2008).

All fate and effect studies required for an ERA should be performed according to international guidelines (e.g., OECD or ISO). In the ERAPharm project, all studies conducted with ivermectin fulfilled this criterion, except for the higher tier studies, i.e., 2-species, multispecies, semifield, and field studies, for which no guidelines are available. In addition, reliable data from the scientific literature were used for the ERA; data quality (reliability) was assessed according to Klimisch et al. (1997). In general, only data considered as reliable were used for the ERA. However, some ERAPharm data included in this paper have recently been submitted for publication and are still being reviewed. Furthermore, it was not in all cases feasible to perform the studies as required by the underlying ERA procedure (VICH 2000, 2004; EMEA 2008). Hence, the presented ERA for ivermectin should be partially regarded as preliminary.

Our present objectives are 1) to conduct an ERA for the parasiticide ivermectin, mainly according to the current guidelines for environmental impact assessment (VICH 2000, 2004; EMEA 2008) but taking several species and various routes of administration into account, and 2) to show gaps and to propose improvements of the existing guidelines by integrating data derived from nonstandardized studies into higher tier risk assessment procedures.

ENVIRONMENTAL RISK ASSESSMENT ACCORDING TO VICH (2000, 2004) AND EMEA (2008)

In phase I, a number of questions concerning application and properties of the VMP direct the ERA to the main exposure scenarios, i.e., aquaculture, intensively reared, or pasture animals (VICH 2000). Then, predicted environmental concentrations (PEC) are estimated based on the dose and frequency of the product applied. If the PEC exceeds the trigger value of 100 mg/kg dry wt in soil for intensively reared and pasture animals, studies on environmental fate and effects on selected nontarget species have to be performed in phase II (VICH 2004). For parasiticides used in treatment of pasture animals, the PEC_{soil} trigger is circumvented, and phase II studies are necessarily independent of PEC_{soil}. In phase II, the environmental risk is characterized deterministically by comparing the PECs with the predicted no effect concentrations (PNECs) in several environmental compartments.

According to the guidelines (VICH 2000, 2004), the initial ERA is based on worst-case assumptions (e.g., with regard to

applied dose, excretion, fate, and behavior in the environment), whereas for further refinements averaged values are used (e.g., K_{OC}), when data allow for averaging. In the present case study, parameters such as DT50 and K_{OC} were derived for different soils, which reflect several European regions and climatic conditions. Because these conditions vary considerably, it was not assumed that data allow for averaging. Consequently, minimum and maximum PECs are shown, demonstrating the possible range of environmental exposure resulting from the use of veterinary medicines containing ivermectin.

PHASE I

According to our knowledge, ivermectin is not currently used in marine aquaculture in Europe. Hence, this scenario is not considered in the present case study. Predicted environmental concentrations of ivermectin in soil (PEC_{soil}) were calculated for the intensively reared (IR) and pasture animal scenarios (P), considering worst-case assumptions (EMEA 2008). All estimated initial PEC_{soil} values were below the action limit of 100 μg/kg dry wt (see *Predicted environmental concentrations* section). However, because ivermectin is administered as an endo- and ectoparasiticide to animals reared on pasture, e.g., cattle and sheep, a phase II assessment is required independent of the PEC_{soil} (VICH 2004). Although not required by VICH (2000), a phase II assessment was also performed in this study for ivermectin administered to intensively reared animals.

PHASE II TIER A

In phase II, the PECs for various environmental compartments are compared to the corresponding PNECs (VICH 2004). If in phase II tier A, a compartment-specific PEC exceeds the organism-specific PNEC, an environmental risk is indicated, and tier B testing for the specific compartment including the organisms of concern is required. Phase II tier A assessment relies on a base set of data on physicochemical properties (Table 1), on environmental fate, and on effects determined in single-species tests under laboratory conditions.

Environmental fate

In ERAPharm, sorption was determined mostly according to OECD 106 (OECD 2000) for artificial and 2 natural loamy soils using ^{3}H-labeled and nonlabeled ivermectin (Table 2) (Krogh et al. 2008). Halley, Jacob, et al. (1989) studied sorption of ivermectin in a clay loam and a silty loam soil (Table 2). Equilibrium distribution was reached within 48 h (Krogh et al. 2008) and 16 h (Halley, Jacob, et al. 1989). The estimated K_d values (average values of sorption and 2 desorption steps) from the latter experiments were 227 and 333 L/kg, corresponding to K_{OC} values of 1.48×10^4 and 1.57×10^4 L/kg, indicating strong sorption (Halley, Jacob, et al. 1989).

In soil column experiments with 2 soils containing 2.3 and 6.3% organic carbon content, no ivermectin was detected in the leachate (Oppel et al. 2004), whereas in another study, 27% to 48% of the applied ^{3}H radioactivity was leached as transformation products, and 39% to 49% remained in the top 5 cm of the soil column (Halley, Jacob, et al. 1989). The identity of this strongly sorbed fraction remained undetermined but was assumed to be mostly the parent substance.

Table 1. Physicochemical properties of ivermectin (CAS 70288-86-7)

Molecular mass (g/mol)	874.7[a]
pKa	Neutral at all pH values
Melting point (° C)	349.8[b] (est)
Vapor pressure (Pa)	$<1.5 \times 10^{-9}$[c] (m)
Henry constant (–)	4.8×10^{-26}[b] (est)
Water solubility (mg/L)	4.0[d] (m), 4.1[e] (m), 2.0[f] (m)
Log K_{OW} (–)	3.2[d] (m)
Log K_{OC} (L/kg)	3.6–4.4[g] (m)
UV-visible absorption spectrum	Maxima: 237, 245 and 253 nm (subject to direct photolysis)[c]

[a]Referring to ivermectin consisting of 94% B_{1a} and 2.8% B_{1b}, which was used in most of the tests performed within ERAPharm.
[b]U.S. EPI-Suite v.4.00 (2008).
[c]Halley, Nessel, et al. (1989).
[d]USFDA (1990). Dossier data, no details on experimental methods are available.
[e]Escher et al. (2008). Determined using a modified shake flask method according to Avdeef et al. (2007).
[f]Escher et al. (2008). Intrinsic solubility determined using a μDISS Profiler™.
[g]Krogh et al. (2008).
m = Measured; est = estimated.

The limited mobility of ivermectin in soils justifies the assumption of little potential for groundwater contamination.

Transformation of ivermectin in soil was investigated in ERAPharm according to OECD 307 (2002a) using nonlabeled ivermectin. The results indicate that dissipation half-lives (DT50) in soil can be rather variable depending on soil type, sorption capacity, temperature, and oxygen availability (Krogh et al. 2009). The highest DT50 of 67 d was derived with a simple first-order model for natural soil at 20°C under aerobic conditions (Table 3). This DT50 was used as a worst-case value in the exposure assessment. Within the study of Krogh et al. (2009), 2 transformation products of ivermectin were identified in soil, a monosaccharide and an aglycone of ivermectin (22,23-dihydroavermectin B_1 monosaccharide and 22,23-dihydroavermectin B_1 aglycone; our observations). However, the transformation products were quantified at levels <10% of the parent compound, so no transformation products were considered in the present ERA.

Literature data from mostly nonstandardized biodegradation tests indicate a broad range of DT50 values resulting in classifications ranging from slightly to moderately persistent in soil (DT50 = 14–56 d) to slightly to very persistent in mixtures of soil and manure or feces (DT50 = 7–217 d; Boxall et al. 2002). Halley, Jacob, et al. (1989) investigated the aerobic transformation of ivermectin in soil–feces mixtures and determined DT50 values of 93 d and 240 d, depending on soil type and mode of application. Reports of low ivermectin persistence in manure following summer or dry conditions might be an artefact resulting from reduced ivermectin extraction efficiency at low moisture content of the solid matrix (Pope 2010).

Degradation of ivermectin in water–sediment systems was investigated within ERAPharm according to OECD 308 (2002b) using natural sediment containing 4.5% total organic carbon (TOC), with resulting compartment-specific degrada-

Table 2. Soil parameters, sorption/desorption properties, and organic carbon normalized adsorption coefficients (K_{OC}) for 5 different soils

Soil type	pH	f_{om}	f_{oc}	K_d (L/kg)	K_{des} (L/kg)	K_{OC} (L/kg)	Log K_{OC}
Artificial (OECD)[a]	6.0	0.047	0.0273	109	141–246	4.00×10^3	*3.6*
York, UK[a]	6.3	0.0265	0.0154	396	54–201	2.58×10^4	*4.4*
Madrid, E[a]	8.7	0.0077	0.0045	57	28–56	1.28×10^4	4.1
Newton, USA[b]	5.5	0.039	0.0226	333[c]	n. d.	1.47×10^4	4.2
Fulton, USA[b]	6.3	0.025	0.0145	227[c]	n. d.	1.57×10^4	4.2

Italicized values were used for best- and worst-case exposure assessment. f_{om} = Fraction of organic matter; f_{oc} = fraction of organic carbon (converted from F_{om} according to Halley, Jacob, et al. 1989); K_d = measured soil–water distribution coefficient; K_{des} = measured desorption coefficient; K_{OC} = organic carbon normalized adsorption coefficient calculated according to $K_{OC} = K_d/f_{oc}$; n.d. = not determined.
[a]Soils investigated within ERAPharm (Krogh et al. 2008), mostly according to OECD 106 (only 0.5 g soil was used and Freundlich isotherms were determined only for 1 soil type).
[b]Clay loam and silty clay loam (Halley, Jacob, et al. 1989); K_{OC} values were recalculated according to Halley, Jacob, et al. (1989): $K_d \times 100/(f_{om}/1.72)$.
[c]Soil/0.01 M $CaCl_2$ partition coefficient, average of sorption and 2 desorption steps (Halley, Jacob, et al. 1989).

tion half-lives ($t_{1/2}$) as shown in Table 3 and an estimated dissipation half-life (DT50) in water of <0.25 d (Prasse et al. 2009). Löffler et al. (2005) also investigated the fate of ivermectin in water–sediment systems. The authors found a dissipation half-life (DT50) of 15 d for the whole system containing natural sediment with 1.4% TOC; the DT50 from the water phase was estimated to be 2.9 d (Löffler et al. 2005). The sediment–water distribution coefficients ($K_{d\ sediment}$) of ivermectin were 160 and 11.7 L/kg, corresponding to K_{OC} values of 3550 and 1172 L/kg, respectively (Löffler et al. 2005; Prasse et al. 2009). In a long-term outdoor aquatic mesocosm study (265 d) with ivermectin using natural water and sediments, a DT50 of 4 d was derived for the water phase. However, no DT50 for sediment could be determined, because after reaching a steady state, no dissipation of ivermectin from the sediment was discernible until the end of the study (Sanderson et al. 2007).

Ivermectin is hydrolytically unstable both in acidic and in basic solution, being most stable at a pH of 6.3 (Fink 1988). Data on hydrolysis in environmental matrices were not

Table 3. Transformation of ivermectin in soils and aquatic sediments

Type of study		Value	Reference
Transformation in soil (OECD 307)[a]			
Dissipation (Madrid soil)	DT50	*16* d	Krogh et al. (2009)
	DT90	54 d	Krogh et al. (2009)
Dissipation (York soil)	DT50	*67* d	Krogh et al. (2009)
	DT90	222 d[b]	Krogh et al. (2009)
Dissipation (artificial soil)	DT50	458 d[b]	Krogh et al. (2009)
	DT90	1520 d[b]	Krogh et al. (2009)
Transformation in water–sediment systems (OECD 308)			
Dissipation: DT50 (water)		*<0.25* d	Prasse et al. (2009)
Dissipation: DT50 (whole system)		127 d	Prasse et al. (2009)
Degradation: $t_{1/2}$ (water)		*30* d	Our calculations based on Prasse et al. (2009)
Degradation: $t_{1/2}$ (sediment)		*130* d	Our calculations based on Prasse et al. (2009)
Degradation: $t_{1/2}$ (whole system)		87 d	Our calculations based on Prasse et al. (2009)
Dissipation: DT50 (water)		2.9 d	Löffler et al. (2005)
Dissipation: DT50 (whole system)		15 d	Löffler et al. (2005)

Italicized values were used for best- and worst-case exposure assessment.
[a]Calculated with simple first order model (OECD 2002a); conditions: aerobic at 20°C (Krogh et al. 2009).
[b]Values above 120 d are extrapolated; the last sampling took place at day 120.

available in the scientific literature. The photolytic half-life of ivermectin determined in a thin, dry film exposed to direct sunlight was approximately 3 h (Halley, Jacob, et al. 1989). Photoinduced reactions are thus anticipated to influence the fate of ivermectin in the aquatic environment. Studies on photolysis and hydrolysis might be required by regulatory authorities based on expert judgement. However, the results of a long-term outdoor aquatic mesocosm study (Sanderson et al. 2007) with ivermectin using natural water and sediment suggest that both processes play a minor role, insofar as ivermectin dissipates rapidly from the water phase into the sediment.

Predicted environmental concentrations

Ivermectin may enter the terrestrial compartment via spreading of manure from intensively reared animals on arable land or by excretion of dung by animals on pastures. Likewise, it can be released directly to surface water via treated animals (e.g., cattle) standing in shallow water bodies. Indirect entry into water might occur via leaching from contaminated soil into groundwater or via runoff from pastures or arable land after application of manure from treated animals. The sediment compartment may be contaminated via transfer from surface waters into sediments or sedimentation of eroded material from pastures or arable land.

Because of its high affinity for soil and particulate matter, neither leaching nor runoff was assumed to be a major source for contamination of freshwater ecosystems with ivermectin (Kövecses and Marcogliese 2005). However, the transport of sorbed ivermectin with eroded soil might be important. The risk of soil translocation from erosion is highest when crop coverage is lowest, i.e., in fall after harvesting or in spring before seeding. The postharvest (and preseeding) period with a high erosion risk coincides with the time when large numbers of animals are treated with ivermectin and farmers are allowed to spread manure (Kövecses and Marcogliese 2005). It may in some regions also coincide with the time of intensive rainfall events, initiating soil erosion.

In Table 4, the initial PECs are shown for those environmental compartments involved in environmental fate and behavior processes relevant for ivermectin. The initial PECs were calculated according to the total residue approach, in

Table 4. Initial PECs of ivermectin in different environmental compartments: soil (PEC_{soil}), groundwater (PEC_{gw}), surface water (PEC_{sw}), and dung (PEC_{dung}), calculated according to EMEA (2008)

Compartment	Unit	PEC	Remark
$PEC_{soil\ initial}$ (IR)[a,b]	µg/kg dry wt	2.61/6.08[c]	Weaner pig (<25 kg), H = 1
		0.63/1.47[c]	Sow with litters, H = 1
$PEC_{soil\ initial}$ (P)[a]	µg/kg dry wt	0.84/2.09[c]	Beef cattle
		0.33	Pony
$PEC_{soil\ plateau}$ (IR)[d]	µg/kg dry wt	2.67/6.22[c]	Weaner pig (<25 kg), H = 1
		0.64/1.50[c]	Sow with litters, H = 1
$PEC_{soil\ plateau}$ (P)[d]	µg/kg dry wt	0.86/2.14[c]	Beef cattle
		0.34	Pony
$PEC_{gw\ initial}$ (IR)	ng/L	3.3–21.5[e]	Weaner pig ($PEC_{gw} = PEC_{porewater}$)
$PEC_{gw\ initial}$ (P)	ng/L	0.5–7.4[e]	Beef cattle ($PEC_{gw} = PEC_{porewater}$)
$PEC_{sw\ initial}$ (IR)	ng/L	0.1–7.2[e]	Sow with litters – weaner pig ($PEC_{sw} = 1/3\ PEC_{porewater}$)
$PEC_{sw\ initial}$ (P)	ng/L	0.2–2.5[e]	Beef cattle ($PEC_{sw} = 1/3\ PEC_{porewater}$)
$PEC_{sw\ initial}$ (P; d.e.[f])	ng/L	209/523[c]	Beef cattle
		83	Pony
$PEC_{dung\ initial}$ (P)	mg/kg dung fresh wt	5.08/12.69[c,g]	Beef cattle
		4.8[g]	Horse

PECs are shown only for those species with highest and lowest values for the respective compartment and scenario. IR = intensively reared animals; P = pasture animals; H = housing factor (fraction of the year in which the animals are kept in house).

[a]Initial $PECs_{soil}$ at 5 cm mixing depth.

[b]Assuming the EU nitrogen spreading limit of 170 kg N/(ha × y).

[c]Calculated with minimum/maximum dose.

[d]PEC_{soil} at steady state considering degradation properties and accumulation of ivermectin in soil.

[e]Range from maximum best-case to maximum worst-case PEC calculated with maximum and minimum K_{OC} value, respectively (Table 2).

[f]PEC_{sw} calculated for the specific scenario of direct excretion (d.e.) into surface waters from pasture animals.

[g]Values based on dry wt: 28.6/71.5 for beef cattle and 27.0 for horse (conversion factor fresh wt/dry wt = 5.63; our results).

which it is assumed that 100% of the total dose administered during the treatment is released to the environment (EMEA 2008). Calculation of PECs is based on different types of dosages (0.1–0.5 mg/kg body wt) and application frequencies (1, 2, or 7 applications) to several productive livestock species. This information was compiled from summaries of product characteristics for ivermectin-containing products (Chanectin®, Diapec®, Ecomectin®). In addition to the maximum PEC values as requested according to EMEA (2008), minimum PEC values are indicated.

For the soil compartment, a range of PECs was derived for the IR and P scenario, with a minimum of 0.33 μg/kg dry wt and a maximum of 6.08 μg/kg dry wt, estimated for ponies and weaner pigs, respectively (Table 4). For persistent compounds ($DT90_{soil}$ >1 y), accumulation in soil after application of manure during successive years is possible, and, hence, a $PEC_{soil\ plateau}$ at steady state should be calculated according to EMEA (2008). Although not required for ivermectin (DT90 = 222 d; see Table 3), the worst-case $PEC_{soil\ plateau}$ was calculated. Because this value, 6.22 μg/kg dry wt, is only slightly above the initial PEC_{soil} of 6.08 μg/kg dry wt, it was not used further in the ERA.

The concentrations of ivermectin in groundwater and surface water were estimated based on the PEC_{soil}, assuming that the concentration in groundwater equals the concentration in soil porewater at a mixing depth of 20 cm. $PEC_{porewater}$ was calculated assuming sorption equilibrium of ivermectin between soil and porewater, characterized by K_d or K_{OC} (Table 2). Using the lowest and highest log K_{OC} values (3.6 and 4.4) and the minimum and maximum PEC_{soil} (0.33 and 6.08 μg/kg dry wt), the predicted groundwater concentrations range from 0.5 to 21.5 ng/L (Table 4). The initial PEC_{sw} is assumed to be one-third of the soil porewater concentrations (EMEA 2008) resulting in initial PEC_{sw} values from 0.1 to 7.2 ng/L.

The specific P scenario of direct excretion by pasture cattle via urine or feces into surface water takes into account a standard pasture of 1 ha containing a shallow, slow-flowing ditch covering 1% of the area. It is assumed that pasture animals excrete 1% of the total dose administered within 1 d directly into the stream (EMEA 2008). For other specific P scenarios proposed by EMEA (2008), e.g., runoff from contaminated hard standing areas, neither a model to calculate the specific PEC nor relevant data were available.

For the dung compartment, initial PECs for application to all target animal species were between 4.8 and 8.0 mg/kg dung fresh wt, except for beef cattle (12.69 mg/kg dung fresh wt). Halley, Nessel, et al. (1989) derived PECs for ivermectin in dung and soil following administration of ivermectin to various livestock in feedlots or on pasture. In contrast to the total-residue approach proposed by EMEA (2008), they assumed constant excretion of the applied dose over a feedlot for a period up to 120 to 168 d. Ivermectin concentrations in feces were estimated to be 18 to 19 μg/kg dung fresh wt for swine, sheep, and cattle. Assuming manure application under good agricultural practice and 15-cm plowing depth resulted in a PEC_{soil} of 0.2 μg/kg dry wt for intensively reared cattle and swine. The estimated application rates for sheep and cattle dung on pasture were 0.013 and 0.016 mg ivermectin/m^2, respectively (Halley, Nessel, et al. 1989). However, Fernandez et al. (2009) and Lumaret et al. (2007) measured ivermectin concentrations of 145 μg/kg dung fresh wt and approximately 250 μg/kg dung fresh wt in cattle dung at the excretion peak, which are much higher than the value estimated by Halley, Nessel, et al. (1989).

Aquatic short-term effect studies

The base set data according to EMEA (2008) on short-term effects of ivermectin to fish, *Daphnia*, and algae from the literature was supplemented with data derived from ERA-Pharm (Garric et al. 2007; Table 5). Within ERAPharm, a growth inhibition test with the green alga *P. subcapitata* exposed to ivermectin was performed according to OECD 201 (2002c). EC50 for yield and growth rate was >4.0 mg/L, and NOEC was 391 μg/L (Garric et al. 2007). Ten *Daphnia* immobilization tests were performed according to OECD 202 (2004a). To avoid photodegradation, these tests were conducted in the dark. EC50 values ranged from 1.2 to 10.7 ng/L (mean value 5.7 ng/L; Garric et al. 2007). These values are

Table 5. Phase II tier A aquatic short-term effect studies

Test organism	Test method	Effect concentration	Reference
Pseudokirchneriella subcapitata (green alga)	OECD 201 (2002c)	*$EC50_{72\ h,\ yield,\ growth\ rate}$ >4 mg/L*[a,b]	Garric et al. (2007)
		$LOEC_{72\ h,\ yield,\ growth\ rate}$ = 1.25 mg/L[a]	
		$NOEC_{72\ h,\ yield,\ growth\ rate}$ = 391 μg/L[a]	
Daphnia magna (crustacean)	OECD 202 (2004a)	$EC50_{48\ h,\ immobility}$ = 1.2–10.7 ng/L[c]	Garric et al. (2007)
		Mean $EC50_{48\ h}$ = 5.7 ng/L (n = 10)[c]	
	USEPA 660/3-75-009 (1975)	$LC50_{48\ h}$ = 25 ng/L[a]	Halley, Jacob, et al. (1989)
Oncorhynchus mykiss (fish)	USEPA 660/3-75-009 (1975)	*$LC50_{96\ h}$ = 3.0 μg/L*[a]	Halley, Jacob, et al. (1989)
Salmo salar (fish)	Acute toxicity test (juvenile fish)	$LC50_{96\ h}$ = 17 μg/L[a]	Kilmartin et al. (1996)

Results of the most sensitive tests (italicized) were used for the risk characterization.
[a]Based on nominal concentrations.
[b]According to VICH (2004), the EC50 is used for risk characterization in phase II tier A.
[c]Based on measured concentrations.

slightly below the LC50 of 25 ng/L derived for *D. magna* by Halley and colleagues (Halley, Jacob, et al. 1989; Halley, Nessel, et al. 1989). As far as is known from scientific literature, acute effects of ivermectin on fish occur in the lower micrograms-per-liter range, with *Oncorhynchus mykiss* as the most sensitive species. In addition to the standard base set of acute-effects data for algae, *Daphnia*, and fish, acute-effects data are available for estuarine and marine crustaceans, mollusks, and other invertebrates. Overall, crustaceans are the most sensitive taxonomic group, showing effect concentrations in the lower nanograms-per-liter range (see, e.g., Davies et al. 1997; Grant and Briggs 1998; Garric et al. 2007).

Terrestrial effect studies

Results of the terrestrial tests from ERAPharm and the literature are summarized in Table 6. As required by VICH (2004), an earthworm reproduction test according to OECD 220/222 (2004b, 2004c) was performed, resulting in an EC50 of 5.3 mg/kg dry wt and an NOEC of 2.5 mg/kg dry wt (Römbke, Krogh, et al. 2010). Because endo- and ectoparasiticides are not considered to be toxic for plants and microorganisms and the trigger value of 100 μg/kg for PEC_{soil} given in phase I was not exceeded by ivermectin, neither a nitrogen transformation nor a plant test is required according to VICH (2004).

Some EU authorities require information on the toxicity to nontarget arthropods for parasiticides for the IR scenario, so collembolan reproduction tests were performed according to ISO 11267 (ISO, 1999). As expected when considering the mode of action of ivermectin and the taxonomic relationship of collembolans to the target organisms, the tests revealed a high sensitivity as shown by the NOEC of 0.3 mg/kg dry wt (Jensen et al. 2003; Römbke, Krogh, et al. 2010). Earthworms and other oligochaetes were less sensitive, with NOECs in the milligrams-per-kilogram range.

Because ivermectin is used to treat livestock on pasture, tests with dung beetles and dung flies are required in tier A. Table 7 summarizes the results of dung fly and dung beetle tests performed within ERAPharm as well as studies described in the literature. The high sensitivity of *Musca autumnalis* to ivermectin was confirmed in a ring test performed to validate the OECD draft guideline (Römbke, Alonso, et al., 2010), where a mean EC50 of 4.65 μg/kg dung fresh wt was determined. In the literature, effect concentrations of 0.5 μg/kg dung fresh wt were reported for the yellow dung fly *Scathophaga stercoraria* when studying morphological changes in adults (Strong and James 1993). However, these specific endpoints are difficult to assess and were not used for risk characterization. With LC50 values of 100 and 176 μg/kg dung fresh wt, the dung beetle *Aphodius constans* reacted less sensitively to ivermectin than dung flies

Table 6. Phase II tier A terrestrial effect studies with soil organisms

Test organism	Test method	Effect concentration[a]	Reference
Eisenia fetida (earthworm)	OECD 222 (2004c) (artificial soil, TOC 3.6%)	$NOEC_{28\ d,\ biomass} = 5.0$ mg/kg dry wt	Römbke, Krogh, et al. (2010)
		$NOEC_{56\ d,\ reprod.} = 2.5$ mg/kg dry wt	
		$EC50_{56\ d,\ reprod.} = 5.3$ mg/kg dry wt	
Eisenia fetida (earthworm)	Subchronic earthworm toxicity test (artificial soil)	$NOEC_{28\ d,\ biomass} = 12$ mg/kg dry wt	Halley, Jacob, et al. (1989)
		$LC50_{28\ d} = 315$ mg/kg dry wt	
Eisenia fetida (earthworm)	OECD 207 (1984) (artificial soil)	$NOEC_{14\ d,\ biomass} = 4$ mg/kg dry wt	Gunn and Sadd (1994)
		$LC50_{14\ d} = 15.8$ mg/kg dry wt	
Enchytraeus crypticus (potworm)	ISO 16387[b], (field soil: TOC 1.6%)	*$NOEC_{28\ d,\ reprod.} = 3.0$ mg/kg dry wt*	Jensen et al. (2003)
		$EC50_{28\ d,\ reprod.} = 36$ mg/kg dry wt	
		$LC50_{28\ d} > 300$ mg/kg dry wt	
Folsomia candida (collembolan)	ISO 11267 (1999) (artific. soil: TOC 3.6%)	*$NOEC_{28\ d,\ reprod.} = 0.3$ mg/kg dry wt*	Römbke, Krogh, et al. (2010)
		$EC50_{28\ d,\ reprod.} = 1.7$ mg/kg dry wt	
Folsomia fimetaria (collembolan)	ISO 11267 (1999) (field soil: total carbon 1.6%)	*$NOEC_{28\ d,\ reprod.} = 0.3$ mg/kg dry wt*	Jensen et al. (2003)
		$EC50_{28\ d,\ reprod.} = 1.7$ mg/kg dry wt	
		$LC50_{28\ d} = 8.4$ mg/kg dry wt	

Results of the most sensitive tests (italicized) were used for the risk characterization.
[a]Effect concentrations refer to nominal concentrations.
[b]The test was performed according to a slightly modified method described by Römbke and Moser (1999) published as ISO 16387 (2004).

Table 7. Phase II tier A terrestrial effect studies with dung organisms

Test organism	Test method	Effect concentration[a]	Reference
Musca autumnalis (dung fly)	OECD (2008a)	*$EC50_{21\ d,\ emergence\ rate}$ = 4.65 μg/kg dung fresh wt*	Römbke, Barrett, et al. (2010)
Scathophaga stercoraria (dung fly)	OECD (2008a)	$LC50_{28\ d}$ = 20.9 μg/kg dung fresh wt	Römbke et al. (2009)
		$NOEC_{28\ d,\ development\ time}$ = 0.84 μg/kg dung fresh wt	
	Specific test design (acute toxicity)	$LC50_{48\ h,\ larvae}$ = 36 μg/kg dung fresh wt	Strong and James (1993)
		$EC50_{3–4\ w.,\ emergence}$ = 1.0 μg/kg dung fresh wt	
Aphodius constans (dung beetle)	OECD draft (2009)	*$LC50_{21\ d}$ = 176 μg/kg dung fresh wt*	Hempel et al. (2006)
		$LC50_{21\ d}$ = 880 μg/kg dung dry wt	
		$NOEC_{21\ d,\ larval\ survival}$ = 320 μg/kg dung dry wt	
Aphodius constans (dung beetle)	OECD draft (2009), modified	$LC50_{21\ d}$ = 100 μg/kg dung fresh wt[b]	Lumaret et al. (2007)
		$LC50_{21\ d}$ = 590 μg/kg dung dry wt	

Results of the most sensitive tests (italicized) were used for the risk characterization.
[a]All effect concentrations refer to nominal concentrations.
[b]Instead of spiked dung as recommended in OECD (2009), dung from treated cattle was used. The resulting EC50 was, thus, not used for phase II tier A risk characterization.

(Hempel et al. 2006; Lumaret et al. 2007). The LC50 of 176 μg/kg dung fresh wt was used for the ERA. A lower LC50 of 100 μg/kg dung fresh wt was derived with dung from treated cattle (Lumaret et al. 2007). This approach is not recommended by OECD (2009) but is considered to be appropriate for higher tier testing, in that it reflects a more realistic exposure scenario.

Large numbers of additional tests with dung fauna species were performed, but most of them have limited value for a quantitative ERA, because NOEC or ECx values were not determined. In particular, only information on mortality in relation to the age of the dung is given in tests with treated dung as test substrate. Because the concentration of ivermectin can hardly be related to the observed effects, these data (e.g., NRA 1998; Steel and Wardhaugh 2002) are not taken into consideration for the risk assessment. In a test with dung-living nematode species, an NOEC of 3.0 mg/kg dung fresh wt was determined (Grønvold et al. 2004), which is higher than the values found for dung flies and beetles, although both insects and nematodes belong to the target organisms of ivermectin.

Risk characterization

Based on the data shown in Tables 4 and 5, the risk quotient (RQ), i.e., the ratio of initial PEC to PNEC, for the aquatic compartment was determined. According to VICH (2004), an assessment factor (AF) of 1000 was applied to the acute effect concentrations for daphnids (EC50) and fish (LC50) and an AF of 100 to the EC50 for algae in order to derive the PNECs (Table 8).

Ivermectin is unlikely to present a risk for freshwater algae. For fish, a PNEC of 3 ng/L was derived based on the lowest LC50. This value is within the range of the initial PEC_{sw}. The RQ using the worst-case PEC_{sw} for the IR scenario is above the threshold of 1, indicating a risk for freshwater fish. For the specific P scenario assuming direct excretion from the treated animals into surface waters, the initial PEC_{sw} values are higher, thus also indicating a risk. The most sensitive aquatic species is the crustacean *D. magna*, with a mean EC50 of 5.7 ng/L (Table 5), which was used to derive the PNEC of 5.7 pg/L. For all scenarios, the RQs indicate a high risk for aquatic invertebrates (Table 8).

To derive PNECs for the terrestrial compartment, the EC50 of the plant and the LC50 of the dung organism toxicity tests are divided by an AF of 100, whereas the NOECs from the chronic earthworm and collembolan toxicity tests are divided by an AF of 10 (Table 9). The most sensitive endpoint for soil organisms was collembolan reproduction. However, the risk quotient between 0.01 and 0.48 did not indicate a risk for soil arthropods. For dung beetles, the LC50 of 176 μg/kg dung fresh wt derived from a test with spiked dung was used for the risk assessment. The resulting RQs range from 2727 to 317 250, indicating a high risk for dung organisms (Table 9).

According to VICH (2004), a risk characterization for sediment is required when the initial RQ for aquatic invertebrates is ≥1, which is the case for ivermectin (Table 8). In applying the equilibrium partitioning model (EMEA 2008), $PNEC_{sediment}$ was 0.0012 and 0.0074 μg/kg dry wt when using the lowest and the highest K_{OC}, respectively (Table 2). Likewise, the initial estimation of $PEC_{sediment}$ was based on the lowest K_{OC} and minimum PEC_{sw} as well as on the highest K_{OC} (Table 2) and maximum PEC_{sw} (Table 4) of ivermectin. The resulting RQs shown in Table 10 are far above 1.

Table 8. Phase II tier A risk assessment for ivermectin in the aquatic compartment

Species	Effect concentration	AF	PNEC	PEC_{sw} (best/worst case)	RQ (best/worst case)
Pseudokirchneriella subcapitata	EC50 >4 mg/L	100	>40 μg/L	0.1/7.2 (IR)	2.5×10^{-6}/1.8×10^{-4} (IR)
				0.2/2.5 (P)	5.0×10^{-6}/6.3×10^{-5} (P)
				83/523 (P; d.e.)	2.1×10^{-3}/1.3×10^{-2} (P; d.e.)
				ng/L	
Daphnia magna	EC50 = 5.7 ng/L	1000	0.0057 ng/L		**18/1263** (IR)
					35/439 (P)
					14561/91754 (P; d.e.)
Oncorhynchus mykiss	LC50 = 3.0 μg/L	1000	3.0 ng/L		0.03/**2.4** (IR)
					0.07/0.8 (P)
					27.7/174 (P; d.e.)

Values in boldface indicate a risk. AF = assessment factor; PNEC = predicted no effect concentration; PEC_{sw} = initial predicted environmental concentration in surface waters (maximum best-case and maximum worst-case values) for intensively reared (IR) and pasture (P) animal scenarios (see Table 4); RQ = risk quotient (PEC to PNEC ratio); d.e. = direct excretion scenario.

Consequently, refinement of $PEC_{sediment}$ and effects testing using sediment-dwelling organisms and spiked sediment is required (VICH 2004; EMEA 2008).

Refinement of PEC estimation

Exposure assessment can be refined by taking into account metabolism, excretion pattern, and biodegradation of the VMP in aquatic systems, soil, and dung. Fernandez et al. (2009) studied metabolism of ivermectin in cattle dung excreted over a period of 31 d after subcutaneous application of a single dose of 200 μg/kg body wt. The peak of excretion was observed 5.6 days postinjection, with 872 μg/kg dung dry wt (145 μg/kg dung fresh wt; Fernandez et al. 2009). During a period of 31 d postinjection, 35% (±10%) of the applied dose was excreted as parent compound. Based on the daily dung production of 3.8 kg dry wt (our measurements made within the project ERAPharm), the fraction of the total applied dose at the peak of excretion was 3.31%. These experimental data on metabolization of ivermectin in cattle dung correspond well to investigations by Cook et al. (1996), who measured excretion peaks between 2.38 and 1.1 mg/kg dung dry wt on

Table 9. Phase II tier A risk assessment for ivermectin in the terrestrial compartment

	Species	Effect concentrations	AF	PNEC	PEC (best/worst case)	RQ (best/worst case)
Soil	*Vicia sativa, Triticum aestivum*	EC50 >10 mg/kg soil dry wt	100	100 μg/kg soil dry wt	0.63/6.08 (IR)	0.006/0.06 (IR)
					0.33/2.09 (P) μg/kg soil dry wt	0.003/0.02 (P)
	Eisenia fetida	$NOEC_{reprod.}$ = 2.5 mg/kg soil dry wt	10	250 μg/kg soil dry wt		0.003/0.02 (IR)
						0.001/0.008 (P)
	Folsomia candida	$NOEC_{reprod.}$ = 0.3 mg/kg soil dry wt	10	30 μg/kg soil dry wt		0.02/0.20 (IR)
						0.01/0.07 (P)
Dung	*Musca autumnalis*	$EC50_{emerg.rate}$ = 4.65 μg/kg dung fresh wt	100	0.0465 μg/kg dung fresh wt	4.8/12.7 (P) mg/kg dung fresh wt	**103 226/273 118 (P)**
	Aphodius constans	LC50 = 176 μg/kg dung fresh wt	100	1.76 μg/kg dung fresh wt		**2727/7210 (P)**

Values in boldface indicate a risk. AF, PNEC, RQ as described for Table 8; PEC = initial predicted environmental concentration in soil or dung for intensively reared (IR) and pasture (P) animal scenarios (Table 4).

Table 10. Phase II tier A risk assessment for ivermectin in the sediment based on equilibrium partitioning (EMEA 2008)

$PNEC_{D.\ magna}$	$PNEC_{sed}$ (best/worst case)	PEC_{sed} (best/worst case)	RQ (best/worst case)
0.0057 ng/L	0.0074/0.0012 μg/kg dry wt	0.02/9.25 (IR)	**2.7/7708 (IR)**
		0.03/3.18 (P)	**4.1/2650 (P)**
		16.7/675 (P; d.e.)	**2257/562 500 (P; d.e.)**
		μg/kg dry wt	

Values in boldface indicate a risk. $PNEC_{D.\ magna}$ = PNEC derived from acute toxicity to *D. magna* (Table 8); $PNEC_{sed}$ = predicted no effect concentration for sediment organisms; PEC_{sed} = initial predicted environmental concentration in sediment for intensively reared (IR) and pasture (P) animal scenarios derived by equilibrium partitioning (EMEA 2008); RQ = risk quotient (PEC to PNEC ratio); d.e. = direct excretion scenario.

days 6 and 8 postinjection. Using a reverse isotope dilution assay, Halley and colleagues (Halley, Jacob, et al. 1989; Halley, Nessel, et al. 1989) found that 39 to 44% of the total radioactivity in feces of ^{3}H-ivermectin-treated steers was the unaltered active ingredient.

Within ERAPharm, 2 potential metabolites of ivermectin were identified in cattle dung: 24-hydroxymethyl-H_2B_{1a} and 3″-O-desmethyl-H_2B_{1a} (Pope 2010). These metabolites were also reported to be the most prominent in cattle and swine liver (Chiu et al. 1986, 1990; Halley et al. 1992). However, the potential metabolites could not be quantified because of time constraints on the preparation of the appropriate metabolite standards. According to the chromatograms, the amount of the metabolites was estimated to be less than the amount of parent compound (Pope 2010). In addition, the more polar degradation products of ivermectin (monosaccharide and aglycone), as detected as transformation products in soil (see above), were shown to be less toxic to daphnids than the parent compound (Halley, Jacob, et al. 1989). Therefore, the PEC refinement taking metabolization and excretion data into account was performed based on the percentage of excreted parent compound (35%).

For pasture animals directly excreting into surface waters (P; d.e.), refined PEC_{sw} and $PEC_{sediment}$ were calculated by taking into account sorption and distribution properties of ivermectin and assuming 100% excretion (EMEA 2008). For beef cattle, considering low (0.2 mg/kg body wt/d) and high doses of ivermectin (0.5 mg/kg body wt/d) and high (4.4) and low (3.6) log K_{OC} values (cf. Table 2), maximum best- and worst-case values for PEC_{sw} were 1.9 and 29.4 ng/L, respectively. Likewise, the best- and worst-case $PEC_{sediment}$ was 0.91 and 2.4 μg/kg sediment wet wt, respectively. Although not proposed by EMEA (2008), the experimentally determined total amount of excreted unchanged ivermectin (35%) was taken into account as a more realistic approach for the PEC refinement. For the P scenario considering direct excretion, this resulted in a worst-case PEC_{sw} of 10.3 ng/L and a worst-case $PEC_{sediment}$ of 0.84 μg/kg sediment wet wt when using the worst-case assumptions for the applied dose and K_{OC} (Table 11).

For the IR scenario, EMEA (2008) recommends use of the FOCUS (2006) models for refinement of PECs for groundwater, surface water, and sediment. The FOCUS groundwater model PEARL is required if the concentration of 0.1 μg/L is exceeded in the metamodel. (The metamodel is an empirical equation fitted to the outcomes of the PEARL model and allows for a rough estimation of PEC_{gw} as a simple function of K_{OC} and degradation half-life $t_{1/2}$ in soil.) Because the metamodel yielded values of <0.1 μg ivermectin/L for the worst- and best-case scenario, running the PEARL model to estimate groundwater concentrations was not considered necessary.

To estimate the long-term exposure concentrations in surface water and sediment, FOCUS requires the degradation half-lives $t_{1/2}$ for ivermectin determined in the water–sediment transformation study (OECD 308; Table 3). According to FOCUS (2006), the best-fit degradation rate constants are $k_w = 0.0229$/d (corresponding to $t_{1/2\ water} = 30$ d) and $k_{sed} = 0.0054$/d (corresponding to $t_{1/2\ sediment} = 130$ d). These data were used in different combinations together with the best-case (16 d) and worst-case (67 d) DT50 in soil (Table 3) to run the FOCUS models. The FOCUS shell SWASH was used to run the 3 models (MACRO, PRZM, and TOXSWA) necessary to calculate contamination of surface water and sediment resulting from runoff and drainage. From the combination of the different FOCUS scenarios (e.g., drainage, runoff) with different water bodies (e.g., pond, stream), 14 scenarios were identified, for which concentration courses were calculated for a period of 1 y. Maximum annual concentrations were 0.77 and 6.2 ng/L in surface water and 0.17 and 0.25 μg/kg wet wt in sediment, assuming best- and worst-case sorption and degradation, respectively (Table 11). For better comparison with PNECs derived from chronic-effects data, additionally time-weighted average (TWA) PECs were calculated using FOCUS for 21, 50, and 100 d, resulting in TWA worst-case PEC_{sw} of 0.7, 0.37, and 0.22 ng/L, respectively.

EMEA (2008) also suggests that VetCalc could be used alternatively to FOCUS (2006). The VetCalc software (Mackay et al. 2005), which was developed specifically for the risk assessment of veterinary pharmaceuticals, offers a wide range of application forms, animal types, and geographic and climatic regions, which can be combined for various scenarios. This results in a large number of potential PECs, so we aimed at simulating worst-case conditions with regard to application form, dosage, animal type, and environmental conditions. Single injections to 2-y-old beef (500 kg body wt) at 0.5 mg/kg body wt were simulated because they resulted in highest PECs and were comparable to FOCUS simulations. For worst-case simulations, we used the lowest K_{OC} and highest DT50 in soil (Tables 2 and 3). We did not consider data on degradation in sediment and water and on excretion, which are included in the software's advanced data section, because use of these data is not recommended by EMEA (2008). This resulted in a worst-case estimate of PEC_{sw} for the P scenario of 12.9 ng/L, which is in the same range as the

Table 11. Refined PECs for ivermectin in surface water, sediment, soil, and dung

				Maximum PEC	
Compartment (scenario)		**Guidance/model (scenario)**	**Unit**	**Best case**	**Worst case**
Surface water	PEC_{sw} (P)	VetCalc[a]	ng/L	0.41	**12.9**
	PEC_{sw} (P; d.e.)	EMEA[b] (sorption + metabolism)	ng/L	0.7	**10.3**
	PEC_{sw} (IR)	FOCUS[c] (runoff scenarios R3 and R4-stream)	ng/L	0.77	6.2
	PEC_{sw} (IR)	FOCUS[d] (TWA for 21, 50, and 100 d)	ng/L	0.1, 0.07, 0.05	**0.70**, 0.37, 0.22
	PEC_{sw} (IR)	VetCalc[a]	ng/L	0.20	**34.7**
Sediment	PEC_{sed} (P; d.e.)	EMEA[b] (sorption + metabolism)	μg/kg wet wt (μg/kg dry wt)[f]	0.32 (0.83)	**0.84 (2.17)**
	PEC_{sed} (IR)	FOCUS[c] (runoff scenario R3-stream)		0.17 (0.45)	**0.25 (0.65)**
Soil	PEC_{soil} (P)	EMEA[b] (metabolism)	μg/kg dry wt	0.12	0.73
	PEC_{soil} (IR)	EMEA[b] (metabolism)	μg/kg dry wt	0.22	2.13
	PEC_{soil} (P)	VetCalc[a]	μg/kg dry wt	1.14	**4.80**
	PEC_{soil} (IR)	EMEA[b] (degradation in manure[g])	μg/kg dry wt	0.44	5.57
	PEC_{soil} (IR)	EMEA[b] (degradation in manure[g] and soil)	μg/kg dry wt	0.47	**11.4**[h]
	PEC_{soil} (IR)	VetCalc[a]	μg/kg dry wt	1.80	10.8
Dung	PEC_{dung} (P)	EMEA[b] (excretion pattern)	μg/kg dung fresh wt (μg/kg dry wt)	159 (894)[e]	**420 (2365)**[e]

Values in boldface are used for refined risk characterizations. P, IR, and d.e. as described for Table 8.
[a]VetCalc software (Mackay et al. 2005).
[b]EMEA (2008).
[c]FOCUS (2006); maximal annual concentrations for the scenario resulting in the highest value.
[d]Maximum time-weighted average (TWA) PECs for 21, 50, and 100 d using FOCUS (2006).
[e]Conversion factor dung fresh wt/dry wt = 5.63 (our results).
[f]Conversion factor sediment fresh wt/dry wt = 2.6 (EMEA 2008).
[g]Since no data for degradation in manure under anaerobic conditions were available, data for degradation in soil–feces mixtures were used (see section Refinement of PEC estimation).
[h]Assuming a scenario of 5 spreading events on grassland with 2-months intervals.

value derived using the EMEA model for the P scenario direct excretion but is higher than the PEC_{sw} (IR) predicted by FOCUS (Table 11). For best-case simulations, we considered the highest K_{OC} and lowest DT50 in soil as well as the advanced data for degradation in sediment and water and on excretion. This resulted in maximum best-case PEC_{sw} of 0.41 and 0.20 ng/L for the P and IR scenarios, respectively. The PEC for groundwater calculated with VetCalc was always 0.000 ng/L during a period of 10 y. It has to be noted that calculations in VetCalc are based on the Leach-P model, which does not simulate particle-bound transport. This means that the best-case PEC_{sw} from VetCalc based on the highest K_{OC} may underestimate the concentration in surface waters.

Although no risk for soil organisms was indicated in phase II tier A (RQ = 0.48; Table 9), a PEC_{soil} refinement was performed according to EMEA (2008) and VetCalc (Mackay et al. 2005), taking into account the excretion pattern and the degradation potential in manure and soil. A simple model provided by EMEA (2008) estimates the refined PEC_{soil} by multiplying the initial PEC_{soil} with the fraction of excreted unchanged ivermectin (≥35%; see above). With this approach, the highest derived values were the refined PEC_{soil} values of 0.73 and 2.13 μg/kg dry wt for the P and IR scenario, respectively (Table 11).

For the PEC_{soil} refinement within the IR scenario, EMEA (2008) provides a further approach, which considers the DT50 of the pharmaceutical in manure, the storage time of manure, and the nitrogen produced during the storage, with default values given for the latter 2 parameters. Furthermore, it is assumed that the EU nitrogen spreading limit of 170 kg N/ha y^{-1} is met by a single spreading event, as is common practice on arable land. Because no data for degradation in manure under anaerobic conditions were available, data for degradation in soil–feces mixtures as specified above (see *Environmental fate* section) were used instead. However, it should be noted that degradation processes in soil–feces mixtures, which normally occur under aerobic conditions, might differ significantly from anaerobic degradation processes in manure or slurry. The highest PEC_{soil} of 11.4 μg/kg dry wt was derived with the worst-case

assumptions of $DT50_{soil/feces} = 240$ d and $DT50_{soil} = 67$ d assuming a scenario of 5 spreading events on grassland with 2-month intervals (Table 11).

Similar to the refinement recommended in EMEA (2008), the framework proposed by Montforts (1999) is used in VetCalc to calculate PEC_{soil}. Based on the combinations of pasture usage, manure management, and environmental scenarios described above, VetCalc predicted maximum PEC_{soil} of 4.80 and 10.8 μg/kg soil dry wt for the worst-case pasture and intensively reared animals scenario, respectively (Table 11).

It should be noted that the worst-case PEC_{soil} derived by the VetCalc and EMEA models resulted on some occasions in higher values than the initial PEC_{soil} derived using the total residue approach (cf. Table 4). Hence, a risk of ivermectin accumulation in soil over time is demonstrated, probably caused by its apparent slow degradation and high adsorption potential.

For the refinement of PEC_{dung}, the highest fraction of the applied dose excreted in 1 d was considered (EMEA 2008). With this fraction (3.31%, see above), the refined maximum PEC_{dung} was 159 and 420 μg/kg dung fresh wt for the lowest and highest dosage (best and worst case), respectively (Table 11). The model provided by EMEA (2008) does not include degradation in dung.

Outcome of phase II tier A refined ERA

Risk quotients were calculated with refined PEC values (Table 11) for those species for which a risk had been indicated based on initial PECs (Table 12). According to the outcome of the phase II tier A refined risk assessment, further effects testing in phase II tier B is required for aquatic crustaceans, fish, sediment-dwelling organisms, and dung organisms. Because the log K_{OC} of ivermectin (3.2; Table 1) is below the trigger value of 4, no potential for bioaccumulation is indicated according to VICH (2004) and, thus, no fish bioaccumulation study was performed. This decision was supported by the fact that bioaccumulation of the closely related avermectin B_{1a} (abamectin) in fish was low: bioconcentration factors (BCFs) of 52 and 56 L/kg were obtained in two 42-d studies with *Lepomis macrochirus* (Wislocki et al. 1989; Van den Heuvel et al. 1996). It was hypothesized that the large molecular size might have led to a reduced membrane permeation and, thus, to a reduced uptake of avermectin B_{1a} by fish.

PHASE II TIER B ENVIRONMENTAL RISK ASSESSMENT

Fate assessment: Semifield level

According to VICH (2004), no further fate studies are required for ivermectin in phase II tier B. However, as part of a semifield study using terrestrial model ecosystems (TMEs), some information on the actual concentrations of ivermectin in soil cores were collected (Förster et al. 2010). The TMEs were designed and performed as described by Knacker et al. (2004). Soil cores were collected from a field site near York, United Kingdom, and established in constant environmental chambers. Ivermectin was applied to the surface of the soil cores via slurry made from spiked cow dung at 7 different concentrations (nominal range 0.75–547 mg/kg soil dry wt, assuming a soil depth of 1 cm and a density of 1.5 g/cm^3). After destructive sampling on days 7, 28, and 96 following application, the concentration of the parasiticide was analyzed in the uppermost 1 cm of the soil cores. At the highest applied nominal concentration, the ivermectin content in soil did not change considerably (36, 27, and 32% of the nominal concentration at 7, 28, and 96 d after application, respectively), whereas, at the second highest applied concentration (182 mg/kg dry wt), the measured contents of ivermectin at the 3 sampling dates were 34, 15, and 21% of the nominal concentration. Ivermectin concentrations in lower soil layers and in lower treatments were below and around the limit of detection of 0.34 μg/kg dry wt (Pope 2010). Given that ivermectin was not directly mixed into the soil but was adsorbed to dung particles applied on the surface of the soil

Table 12. Phase II tier A risk assessment of ivermectin for the most sensitive taxa using refined maximum PECs

	Species	Unit	PNEC	PEC	RQ
Surface water	*Daphnia magna*	ng/L	0.0057	12.9 (P)	**2263**
				34.7 (IR)	**6088**
	Oncorhynchus mykiss	ng/L	3.0	12.9 (P)	**4.3**
				34.7 (IR)	**11.6**
Sediment	*Daphnia magna*	μg/kg wet wt	0.0074–0.0012	0.84 (P; d.e.)	**114–700**
				0.25 (IR)	**33.8–208**
Soil	*Folsomia candida*	μg/kg dry wt	30	4.80 (P)	0.16
				11.4 (IR)	0.38
Dung	*Musca autumnalis*	μg/kg dung fresh wt	0.047	420 (P)	**8936**
	Aphodius constans		1.76	420 (P)	**239**

Values in boldface indicate a risk. RQ, d.e. as described for Table 8; PNEC = predicted no effect concentration (Tables 8 and 9); PEC = refined predicted environmental concentration derived for pasture (P) or intensively reared (IR) animals using different models (Table 11).

cores, these data support laboratory results indicating a low degradation of this compound in soil.

The fate of ivermectin was also assessed in an aquatic semifield mescocosm study (Sanderson et al. 2007). The parasiticide was added to the water column, and concentrations in water and sediment were monitored over time. Ivermectin was found to dissipate rapidly from the water column with a dissipation half-life between 3.1 and 5.3 d. Dissipation was attributed to partitioning of ivermectin into sediment and degradation, probably resulting from photolysis. Analysis of the sediment indicated that, once in the sediment layer, ivermectin was very persistent, with a half-life of >265 d.

Fate assessment: Field level

For the dung compartment, further studies with dung organisms were conducted on the field level (P scenario), because after PEC refinement in tier A, the risk quotient for dung fauna was still ≥1 (Table 12). In these studies, fate of ivermectin was also investigated, despite the fact that this is not explicitly required by VICH (2004).

Two large field studies were performed within ERAPharm in North England and Central Spain, in order to cover the geographic and climatic diversity of Europe. These studies explored exposure by excretion and field degradation of ivermectin in dung from treated cattle. At various intervals (e.g., 21, 14, 7, 5, 3, 1 d) before placing dung pats on the pasture, 4 cattle were treated with ivermectin applied subcutaneously at the recommended dose of 0.2 mg/kg body wt. Six untreated cattle served as the control. Dung pats on the pasture were protected from disturbance by fences and nets.

The first study performed near York, United Kingdom, focussed on excretion rates and persistence of ivermectin as well as on degradation in dung and movement from dung to soil. The concentrations found in dung samples and soil below dung pats are well within the range determined at farm sites in England (Boxall et al. 2006). Almost no transport from the dung to the soil (0-1 cm depth) was observed. There was no apparent degradation of ivermectin (at ~1.3 mg/kg dung dry wt) within the duration of the study (38 d), confirming that this substance is highly persistent in dung under field conditions (Pope 2010).

Similar results were reported from the second field study, which was performed close to Madrid, Spain. Twenty-eight d after deposition, a maximum ivermectin concentration of 4.0 μg/kg soil dry wt was found in the uppermost 2 cm below the dung pats, which contained maximum ivermectin concentrations of 90 to 110 μg/kg dung fresh wt (corresponding to ~450-550 μg/kg dung dry wt) and considerably less (<1.4 μg/kg dry wt) in the layer of 2- to 5-cm depth (Römbke, Barrett, et al., 2010). These results confirm the conclusions derived from laboratory fate studies.

The present results on slow degradation in dung agree with previous field studies. Suarez et al. (2003) estimated a DT50 of up to 180 d in cattle dung (180 d after deposition of the dung pats, 10–57% of the initially applied ivermectin concentration was detected), whereas Sommer et al. (1992) observed no biodegradation during 45 d. These data indicate that slow degradation in soil–dung mixtures can be expected.

One route of entry of topical ectoparasiticides to the aquatic environment that is described by EMEA (2008) is runoff from farmyard hard standing areas. However, no models are currently available for addressing this route of exposure. Field studies were performed on 2 farms in order to quantify the potential concentrations of ivermectin entering aquatic systems via runoff. On the first farm, ivermectin was applied to cattle as a pour-on treatment on 2 occasions. On the second farm, ivermectin was given to sheep as an oral drench on 2 occasions. After each treatment, the runoff behavior of ivermectin was explored over time. Maximum concentrations in runoff following the 2 treatments at the cattle farm were 85.4 and 4.1 ng/L, whereas at the sheep farm, maximum runoff concentrations for the 2 treatments were 120.4 and 28.8 ng/L (Sinclair et al. 2008). Using a proposed factor of 10 for dilution of the runoff in receiving waters, a maximum surface water concentration of 12 ng/L arising from runoff from farmyard hard standing areas was estimated. This is within the range of worst-case PECs derived for P and IR scenarios using the recommended models (Table 11).

Effect assessment: Aquatic and sediment compartment

In phase II tier B, 2 *D. magna* reproduction tests were performed within ERAPharm according to OECD 211 (1998). Because of the analytical limit of quantification for ivermectin of 1 ng/L, only samples from the highest test concentration (1 ng/L) of one of the tests were analyzed, in which recoveries of 70 to 120% were measured. Only the lowest tested concentration did not cause any effects on *D. magna* growth and reproduction, resulting in an LOEC of 0.001 ng/L and an NOEC of 0.0003 ng/L (nominal concentrations; Garric et al. 2007). Thus, acute to chronic ratio (ACR) for *D. magna* was 19 000 (Table 13), which suggests further chronic testing using more realistic exposure conditions and additional taxonomic groups.

Although the risk characterization in tier A indicated a risk for freshwater fish, no further fish testing was performed, considering the much higher sensitivity of daphnids. To our knowledge, no long-term effects data for fish after water exposure to ivermectin are available. However, Johnson et al. (1993) investigated long-term toxicity of ivermectin to 4 fish species after dietary exposure over 50 d. Although the fish species differed in their ability to tolerate ivermectin, no mortality occurred at the lowest dose of 50 μg/kg fish administered every other day. These results suggest that, as expected based on the mode of action of ivermectin, fish are considerably less sensitive than invertebrates.

Toxicity tests were performed with the nematode *Caenorhabditis elegans* in water-only and water–sediment test systems according to ISO (2008) and with *L. variegatus* and *Chironomus riparius* in water–sediment test systems according to OECD 218 (OECD 2004d). For *C. elegans*, reproduction was the most sensitive endpoint resulting in NOECs of ≤1.0 μg/L in the water-only and 100 μg/kg sediment dry wt in the water–sediment test (Table 13). The toxicity test with *C. riparius* was performed using spiked artificial sediment. *Urtica* powder was added to the sediment before application of ivermectin; no additional feeding was provided during the test. An overall NOEC of 3.1 μg/kg sediment dry wt was derived, with dry wt (growth) of the larvae as most sensitive endpoint. In the toxicity test with *L. variegatus*, spiked artificial sediment was used for exposure and *Urtica* and cellulose powder as food source. At concentrations ≥500 μg/kg sediment dry wt, ivermectin had a significant effect on

Table 13. Phase II tier B aquatic and sediment effect studies

	Test organism	Test method	Effect concentration[a]	Reference
Water	*Daphnia magna*	OECD 211 (1998)	*$NOEC_{21\ d,\ reprod.}$ = 0.0003 ng/L*	Garric et al. (2007)
	Caenorhabditis elegans (nematode)	ISO/CD 10872 (2008) (water-only exposure)	$NOEC_{96\ h,\ reprod.} \leq 1.0$ μg/L	This study
Sediment	*C. elegans*	ISO/CD 10872 (2008) (sediment exposure)	$NOEC_{96\ h,\ reprod.} = 100$ μg/kg dry wt	This study
	Chironomus riparius (insect larvae)	OECD 218 (2004d)	*$NOEC_{10\ d,\ larval\ growth}$ = 3.1 μg/kg dry wt*	Egeler et al. (2010)
	Lumbriculus variegatus (benthic oligochaete)	OECD 225 (2007)	$NOEC_{28\ d,\ reprod.,\ biomass} = 160$ μg/kg dry wt	Egeler et al. (2010)
	Benthic communities	Natural sediments and overlying water (224 d)[b], abundance and community composition	meiofauna community: $NOEC_{224\ d} = 6.2$ μg/kg sedim. dry wt Nematodes community: *$NOEC_{224\ d}$ = 0.6 μg/kg sediment dry wt*	Brinke et al. (2010)
Water–sediment	*D. magna, C. riparius*	Two-species study (51 d)[b], abundance and biomass (*D. magna*), survival, growth and emergence (*C. riparius*)[c]	*D. magna*: *$NOEC_{survival,\ biomass}$ = 53 μg/kg dung dry wt* *C. riparius:* *$NOEC_{larval\ survival,\ larval\ growth,\ emergence}$ = 263 μg/kg dung dry wt*	Schweitzer et al. (2010)
	Cladoceran community	Aquatic mesocosm (265 d)[b], abundance and species richness	$NOEC_{10–97\ d,\ species\ richness} < 30$ ng/L[d]	Sanderson et al. (2007)

Results of the most sensitive tests (italicized) were used for the risk characterization.
[a]All effect concentrations refer to nominal concentrations.
[b]Test duration.
[c]Application of ivermectin with spiked dung.
[d]Significant effects were observed at the lowest nominal concentration (30 ng/L). Measured concentrations (d 10–97) were below the detection limit of 1 ng/L.

survival and reproduction and total biomass of *L. variegatus* (Egeler et al. 2010).

In addition to the requirements of EMEA (2008), effects of the parasiticide on the community level were investigated in an indoor microcosm using sediment (0.15% TOC) from a freshwater habitat in Germany with indigenous benthic communities (Brinke et al. 2010). The sediment was spiked with 0.6, 6.2, and 31 μg ivermectin/kg dry wt. After 7, 14, 28, 56, 112, and 224 d of exposure, abundance and composition of the meiofauna were assessed. The effect of ivermectin on free-living nematodes, as part of the meiofauna community, was investigated at the species level. Results were analyzed with univariate and multivariate methods, and principle response curves were fitted and statistically tested with Monte Carlo permutation. NOECs of 6.2 and 0.6 μg/kg sediment dry wt were derived for the meiofauna and the nematode communities, respectively (Table 13).

To simulate direct excretion from pasture animals into surface waters, a 2-species test using a water–sediment test system was performed, in which ivermectin was applied via dung, and long-term effects (51 d) on *D. magna* and *C. riparius* were evaluated (Schweitzer et al. 2010). Chironomid larvae and daphnids were exposed via cattle dung spiked with ivermectin (11, 53, 263, and 1314 μg/kg dung dry wt). The highest ivermectin concentration corresponds to the typical maximum concentration in dung a few days after topical application to cattle (Lumaret et al. 2007). For the chironomids, an overall NOEC of 263 μg ivermectin/kg dung dry wt was derived. With an NOEC of 53 μg ivermectin/kg dung dry wt, the daphnids were slightly more sensitive (Table 13). At all tested concentrations, ivermectin could not be detected in the water phase (limit of quantification 1 ng/L).

The high toxicity to cladocerans was confirmed in a long-term (265 d) aquatic mesocosm study (Sanderson et al. 2007). At the lowest nominal ivermectin concentration of 30 ng/L, cladoceran species richness, the most sensitive endpoint of this study, was significantly affected between d 10 and 97. Copepod species richness and abundance of Ephemeroptera were significantly affected at some but not all sampling dates during the study period. Measured ivermectin concentrations in the water phase during this period were initially about 6 ng/L but dropped to below the detection limit (1 ng/L); concentrations in sediment were about 25 ng/kg sediment fresh wt. Full recovery of the cladoceran and copepod community and of abundance of Ephemeroptera was observed during the following spring, on days 229 and 265 of the study.

Effect assessment: Terrestrial compartment

Although no further guidance on phase II tier B effects testing with soil arthropods is provided by VICH (2004),

laboratory tests with additional species as well as semifield and field studies are mentioned by EMEA (2008) as possible further procedures. Although not required according to the outcome of the ERA performed so far for ivermectin, additional effect studies with soil organisms at the laboratory and semifield levels were carried out to verify the above-mentioned guidance documents and to make a profound assessment of the effects of ivermectin on terrestrial organisms.

At the laboratory level, a chronic test with the predatory mite *Hypoaspis aculeifer* was performed in artificial soil according to a recently developed guideline (OECD 2008b). After 16 d of exposure, reproduction of the mites was affected, with an EC50 of 17.8 mg/kg soil dry wt (Table 14; Römbke, Krogh, et al. 2010).

At the semifield scale, 3 methods with different levels of complexity were used. Two of them are classified as gnotobiotic, i.e., test systems are prepared using sieved soil and introduced test organisms: the MS·3 multispecies soil system (Boleas et al. 2005) and the SMS soil multispecies system (Cortet et al. 2006), whereas the terrestrial model ecosystems (TMEs) are undisturbed soil cores, i.e., soil structure as well as the local soil organism community have not been changed (Knacker et al. 2004). The MS·3 multispecies soil system combines the toxicity assessment of soil and leachates. The leachate toxicity on *D. magna* was the most sensitive endpoint (data not shown; to be published elsewhere). However, this endpoint cannot be directly incorporated into the soil risk assessment and requires a targeted assessment (Tarazona et al. 2010). In the TME study, ivermectin was applied via slurry to soil cores from a field site near York that were kept in constant environmental chambers (see *Environmental fate* section). No effects of ivermectin were found at the tested concentrations on soil respiration and the numbers of nematodes and enchytraeids. The endpoint affected most strongly was the change in the microarthropod community. Detailed results of this study will be published elsewhere (Förster et al. 2010). These results confirm that ivermectin is affecting arthropods more strongly than other soil organism groups, as had already been concluded from the laboratory tests.

Results from the SMS test system have not yet been published. However, as in the laboratory tests, all collembolans were clearly more sensitive than the predatory mite (data not shown; to be published elsewhere). In a similar test with only 2 species, *F. fimetaria* and *H. aculeifer*, the EC10 for the collembolan was even lower (0.02 mg/kg soil dry wt; Jensen et al. 2009; Table 14).

In the 2 field studies performed in York and Madrid, dung was collected after treatment of cattle with ivermectin (see *Fate assessment: Field level* section). After homogenization, standardized dung pats (0.5 kg wet wt, 15 cm diameter) were placed randomly on the meadow sites. Effects on abundance of dung organisms and soil invertebrates below the dung pats as well as dung decomposition were studied for up to 3 months after the start of the test. In both studies, abundance of dung flies was strongly impacted. The number of dung-inhabiting beetles was initially reduced but reached control levels again at later sampling dates (Table 14). No effects were found on abundance of soil microarthropods, which probably is due to the low concentrations of ivermectin found below treated dung pats (<0.001–0.005 mg/kg soil dry wt). Decomposition of dung pats was affected at the Madrid site at a level of 780 μg/kg dung dry wt (Römbke, Barrett, et al., 2010; cf. Table 9).

Use of short-term vs. long-term PECs for surface water

For the P scenario, the refinement of PEC_{sw} according to the EMEA models considers sorption properties and data on metabolism of the pharmaceutical (EMEA 2008). The factor time is not considered in these models, and a basic assumption is that the total residue of unchanged parent compound is excreted within 1 d. However, in the risk characterization of phase II tier B, the refined PEC_{sw} is compared with the PNEC derived from chronic effects data.

The highest fraction of ivermectin is excreted within the first days, with a maximum of 3.31% on day 5 after application to cattle (see *Refinement of PEC estimation* section). With this value for the scenario direct excretion (d.e.), a transient exposure peak for surface waters (short-term $PEC_{sw\ d.e.}$) can be calculated, when refining the default value for the fraction of the total absorbed dose excreted into the stream (*Fe*) of 0.01 (EMEA 2008). This refinement results in a best-case and worst-case short-term $PEC_{sw\ d.e.}$ of 0.06 and 1.0 ng/L, respectively. This short-term PEC could then be

Table 14. Phase II tier B terrestrial effect studies

	Test organism	Test method	Effect concentration[a]	Reference
Soil	*Hypoaspis aculeifer* (predatory mite)	OECD (2008b) (artificial soil, TOC 3.6%)	$NOEC_{reprod.}$ = 3.2 mg/kg dry wt $EC50_{reprod.}$ = 17.8 mg/kg dry wt	Römbke, Krogh, et al. (2010)
	Folsomia fimetaria, H. aculeifer (collembolan, mite)	2-species test system (21 d[b], reproduction)	*F.* fimetaria: $EC10_{reprod.}$ = 0.02 mg/kg dry wt *H. aculeifer*: $EC10_{reprod.}$ = 0.04 mg/kg dry wt	Jensen et al. (2009)
Dung	Wildlife communities: dung beetles, dung flies	Field study Madrid: Abundance, dung decomposition (86 d)[b]	$NOEC_{beetles}$ = 0.81 mg/kg dung dry wt[c] *$NOEC_{flies}$ <0.31 mg/kg dung dry wt*[c] *$NOEC_{decomp.}$ <0.78 mg/kg dung dry wt*	Römbke, Barrett, et al. (2010)

Results of the most sensitive tests (italicized) were used for the risk characterization.
[a]Effect concentrations refer to nominal concentrations.
[b]Test duration.
[c]Abundance of beetles and flies was investigated during the first 28 d of the study.

compared with a PNEC derived from acute toxicity data, in the case of ivermectin, the EC50 for *D. magna* (Table 8).

Screening for PBT properties

Persistent, bioaccumulative, and toxic (PBT) as well as very persistent and very bioaccumulative (vPvB) substances are of particular concern, because their effects are difficult to reverse and are often not detected at an early stage. Therefore, EMEA (2008) suggests assessing these substance properties according to the technical guidance document on ERA of industrial chemicals and biocides (EC 2003). According to the data indicted in Tables 3 and 13, ivermectin fulfills the P criterion (degradation half-life >120 d in freshwater sediment) and the T criterion (chronic NOEC <0.01 mg/L) as indicated in EC (2003). Concerning the bioconcentration factor (BCF) and, thus, the B criterion (BCF >2,000), no measured data are available for ivermectin. By using the simple formula provided by EMEA (2008), a BCF of 100 is estimated based on the K_{OW}. This formula tends to overestimate the BCF for substances with a molecular weight above 700 g/mol, but it can be used to derive an initial worst-case estimate (EMEA 2008). Note that the reliability of the available K_{OW} could not be checked (see Table 1). Given the dissipation and sorption properties of ivermectin (Tables 2 and 3), it is assumed that accumulation in sediment and sediment-dwelling organisms may occur and, hence, that biomagnification processes additionally play a role in the aquatic environment. It should be noted that, according to the guidance on PBT assessment for the implementation of REACH (ECHA 2008), accumulation in soil and soil-dwelling organisms also has to be assessed. Therefore, further studies are required for a reliable PBT assessment of ivermectin, e.g., the determination of the K_{OW} (D_{OW}) according to OECD 107 or 117 and the assessment of the BCF (BAF) for water, sediment, or soil.

Risk characterization

The effects data derived according to and beyond the requirements of VICH (2004) and the maximum refined PECs (Table 11) were used for risk characterization (Table 15). Because long-term effects data are available for at least 3 trophic levels within the respective compartments, an AF of 10 was generally applied to the lowest NOEC values to derive the PNEC according to EMEA (2008). An AF of 1000 was applied to the short-term effects data for *D. magna*. In this case, the PNEC was compared with the short-term PEC as described in the previous section. This risk characterization resulted in a high acute risk indicated for *D. magna* when exposed to water concentrations that might occur transiently during the peak excretion of ivermectin by cattle on pasture. For the field study, no AF was applied to the NOECs for dung organisms, because no guidance for this is given by EMEA (2008).

The risk characterization using long-term effects data for aquatic and sediment organisms (*D. magna* and C. *riparius*) as required according to VICH (2004) resulted in an indication

Table 15. Summary of phase II tier B risk assessment for ivermectin for different compartments

	Species or biological parameter	Effect concentration[a]	Unit	AF	PNEC	PEC	RQ
Surface water	*D. magna*	5.7[b]	ng/L	1000	0.0057	1.0 (P; d.e.)[c]	**175**
	D. magna	0.0003		10	0.00003	12.9 (P)	**4.3×10^5**
						10.3 (P; d.e.)	**3.4×10^5**
						0.70 (IR)[d]	**2.3×10^4**
	D. magna (2-species)	<1		10	<0.1	10.3 (P; d.e.)	**>103**
Sediment	*C. riparius*	3.1	μg/kg sed. dry wt	10	0.31	2.17 (P; d.e.)	**7**
						0.65 (IR)	**2.1**
	Benthic communities	0.6			0.06	2.17 (P; d.e.)	**36**
						0.65 (IR)	**10.8**
Soil	*F. fimetaria* (2-species test)	20[e]	μg/kg soil dry wt	10	2	4.80 (P), 11.4 (IR)	**2.4, 5.7**
Dung	Dung fly community (field)	<0.31	mg/kg dung dry wt	[f]	<0.31	**2.365** (P)	**>7.6**
	Dung decomposition (field)	<0.78			<0.78		**>3.0**

Values in boldface indicate a risk. AF, PNEC, RQ, and d.e. as described for Table 8; PEC = refined maximum predicted environmental concentration derived for pasture (P) or intensively reared (IR) animals as shown in Table 11.

[a]NOEC values (long-term) as shown in Table 13 and 14; one exception is marked with ''c.''

[b]Short-term EC50 as shown in Table 8.

[c]Short-term $PEC_{sw\ d.e.}$ (see section *Use of short term vs. long term PECs for surface water*).

[d]As a more realistic approach, the TWA PEC_{sw} for 21 d (Table 11) was used.

[e]Refers to EC10.

[f]No guidance on assessment factors for such field studies is available. However, even without any AF, a risk is indicated for dung insects.

of risk for these compartments. While the RQ for sediment organisms was between 2.1 and 36, the RQ for daphnids was $>10^5$, indicating a very high risk for aquatic invertebrates. The aquatic 2-species study using dung spiked with ivermectin also resulted in a risk indication for daphnids. For sediment organisms, the risk demonstrated in phase II tier B assessment was confirmed for both the IR and the P scenarios using the effects data from the study with natural benthic communities (Table 15).

For soil, a risk was now indicated for the P and IR scenarios based on the effects on collembolans observed in the terrestrial 2-species study. Based on the results of the field studies, mainly the Madrid study, RQs for dung organisms and dung decomposition were above 1 (Table 15), but no risk was indicated for soil invertebrates. Hence, the phase II tier B risk assessment for ivermectin indicated risk for the compartments surface water, sediment, soil, and dung.

DISCUSSION

The ERA of ivermectin, which was performed mainly according to VICH (2000, 2004) and EMEA (2008), initially resulted in an indication of risk for surface water, sediment, and dung (Table 16, phase II-A). For the aquatic compartment, this risk was based mainly on the extremely high toxicity of ivermectin to daphnids, with long-term effects in the low picograms-per-liter range and a PNEC in the femtograms-per-liter range. Although a risk was also indicated for fish in phase II tier A and hence chronic fish testing was required in tier B according to EMEA (2008), further phase II tier B studies, such as a fish early life-stage test (OECD 1992), were not performed, given the much higher sensitivity of daphnids. Thus, the phase II tier B ERA for aquatic species is based on daphnids only.

For sediment, a risk was indicated at all tiers of the ERA when using data from standardized single-species toxicity tests with sediment-dwelling organisms and from a mesocosm study with natural benthic communities. Furthermore, and beyond the assessment according to the VICH and EMEA guidelines, a high acute risk for *D. magna* was also indicated when comparing the PNEC derived from *Daphnia* short-term effects data with the short-term PEC that might occur transiently during peak excretion of ivermectin by cattle kept on pasture. Because the persistency (P) and toxicity (T) criteria (EC 2003) for ivermectin are fulfilled, and dissipation and sorption properties suggest that bioaccumulation and biomagnification processes may play a role for ivermectin in the aquatic environment, further studies regarding the B property are necessary for supporting the PBT assessment of ivermectin.

Within the present case study, it was not in all cases feasible to perform the studies requested by VICH (2000, 2004) and EMEA (2008): no data were generated regarding octanol–water partitioning, degradation in manure, or effects on fish early life stages. These data gaps increase the degree of uncertainty for some parts of the ERA. In addition, the literature data had to be used in a few cases for which no assessment of reliability was feasible. For example, the only available measured K_{OW} was taken from dossier data (USFDA 1990), for which no details on the experimental method are available. Furthermore, some data used in the ERA were recently submitted for publication and are still being reviewed. These facts further contribute to the uncertainty of the present ERA for ivermectin, which should therefore partially be regarded as preliminary. However, each risk assessment suffers from some degree of uncertainty regarding the available data, the extrapolation, or the risk characterization. For this reason, assessment factors were employed at all tiers of the ERA.

The environmental concentrations used in the risk assessment are predicted values using models, several of them provided by EMEA (2008), all of which offer a number of choices to the applicant on how to parameterize the models. This leads to a range of PECs resulting in best- and worst-case risk characterizations but also to a higher level of uncertainty. It would be helpful to have more detailed guidance on how these risk characterizations should be systematically evaluated, reported, and interpreted, and also which scenarios should be chosen (Schneider et al. 2007). This issue is critical if RQs are close to 1 and model parameterization, choice of application mode, and exposure scenarios have an important effect on exposure concentrations.

A limited amount of monitoring data is available with maximum ivermectin concentrations in the water column of ditches in the pasture environment of <0.2 ng/L (Boxall et al. 2006). This suggests that concentrations in surface waters

Table 16. Overview of the overall risk assessment for ivermectin according to the tiered approach recommended by VICH (2000, 2004) and EMEA (2008) and additional studies performed within the present case study

	Organism	Phase II-A initial PEC	Phase II-A refined PEC	Phase II-B refined PEC
Surface water	Algae	No risk	Not required	Not required
	Daphnia	Risk	Risk	Risk
	Fish	Risk	Risk	No data available
Sediment	Chironomids and benthic communities	Risk	Risk	Risk
Soil	Plants	No risk	Not required	Not required
	Earthworms	No risk	Not required	Not required
	Collembolans	No risk	Not required	Not required, but risk in 2-species study
Dung	Dung beetles and dung flies	Risk	Risk	Risk (field study)

might be lower than the models predict. However, high toxicity of ivermectin to daphnids was observed at concentrations clearly below the detection limit for this compound in water.

Previous ERAs of products containing ivermectin had revealed no concern for the aquatic compartment (e.g., USFDA 1996, 2001). This was based on the fact that the high sorption and low leaching potential of ivermectin had suggested little potential of exposure of aquatic species. However, Garric et al. (2007) showed that extremely low ivermectin concentrations, which can be expected despite the sorptive properties of the parasiticide, may cause effects on daphnids. Moreover, the additional aquatic 2-species study simulating direct excretion into surface water (Schweitzer et al. 2010) confirmed the risk for daphnids. For the soil compartment, the risk assessment in phase II tier A according to VICH (2004) and EMEA (2008) did not reveal a risk, whereas the terrestrial 2-species study, which was performed beyond the requirements of the guidelines, indicated a risk for collembolans.

In USFDA (2001), transient effects of ivermectin on dung-insect populations were regarded as not relevant for the environment, assuming rapid degradation of ivermectin in sunlight. However, the field studies performed within ERAPharm showed that ivermectin poses considerable risk to dung fauna and dung decomposition. The field studies may have overestimated the risks for dung organisms, because farmers are usually only treating animals for a few days each year. Hopefully, in the near future, improved management practice will lead to a more targeted treatment of livestock parasitosis and, thus, to a reduction of effects on dung organisms. However, further research is needed to improve the understanding of the interactions among infectious diseases caused by parasites, the life cycle of dung organisms, and the possible impact of parasiticides on the dung fauna as well as livestock management and the veterinary practice to treat such diseases. One possible approach to extrapolate results of laboratory and field studies to the actual agricultural situation might be to employ population-modeling approaches together with information on ivermectin usage, excretion characteristics, and animal husbandry methods as used by Boxall et al. (2007). It should be noted that new concepts for higher tier dung-fauna studies were discussed recently with dung fauna experts including long-term laboratory tests with sublethal endpoints (Adler and Römbke 2008).

For both the IR and the P scenario, initial PEC_{soil} values for ivermectin were below the trigger value (action limit) of 100 μg/kg soil dry wt. According to VICH (2000), the ERA of ivermectin-containing products applied exclusively to intensively reared animals stops after phase I, because concentrations below the trigger value are not expected to result in risks for the environment following the IR animal exposure scenario (see also Schmitt et al. 2010). However, for antiparasitic products intended for animals reared on pasture, phase II testing is required independently of the predicted environmental exposure concentration. The risk assessment presented in this paper clearly demonstrates that, for both the IR and the P scenario, an unacceptable risk is determined for all investigated compartments (surface water, sediment, soil, and dung). Hence, the action limit of 100 μg/kg soil dry wt is not protective for substances such as ivermectin used on intensively reared animals. Possible alternatives to the action limit are discussed by Schmitt et al. (2010).

The refined PEC_{soil} values for ivermectin in the IR and P scenarios, which integrate information on adsorption, degradation, and excretion, were by factors of 1.9 and 2.3 higher, respectively, than the initial PEC_{soil}. At phase I, the tiered approach of VICH (2000) and EMEA (2008) does not consider properties of the active ingredient, which might result in potential for accumulation in soil (at this stage, only degradation in manure can be considered, as far as such data are available, to reduce the initial PEC_{soil}). In consequence, exposure to compounds with high-adsorption and low-degradation properties can be underestimated using the initial PEC_{soil}.

Data from the literature (e.g., Madsen et al. 1990; Floate 1998a, 1998b; Krüger and Scholtz 1998a, 1998b; Lumaret and Errouissi 2002) as well as from studies of structural and functional endpoints within ERAPharm show that higher tier evaluation of effects under field conditions provides information essential for the ERA. Despite the large amount of data, regulatory guidance on how to conduct field studies is not yet available. Nevertheless, it can be concluded that the decomposition of dung is a promising parameter for assessing the impact of parasiticides on ecosystem function and services (Millennium Ecosystem Assessment 2003; Svendsen et al. 2003). In addition, the dominance spectrum or species number of soil or dung communities might also be relevant endpoints. To date, no clear criteria or plausible recommendations are available for a tiered effects assessment in the dung compartment. Because these issues have successfully been addressed in aquatic ecotoxicology (see, e.g., Giddings et al. 2002), it should also be possible to provide suitable guidance for the terrestrial compartment. Finally, research is needed to check which scale of field studies (in ERAPharm studies up to 1 ha) is appropriate, insofar as larger scales probably are required for studying issues such as the recovery of dung organisms.

Based on the outcome of the ERA, risk-mitigation measures may be necessary to avoid the possible entry of ivermectin into the environment. The requirement and definition of risk-mitigation measures within the registration and authorization procedures for veterinary pharmaceuticals is a common practice (Koschorreck and de Knecht 2004). However, different entry pathways resulting from different application methods have to be considered, and measures have to be specifically tailored. Therefore, further research is needed to identify appropriate risk-mitigation measures for ivermectin containing veterinary medicinal products. It may be appropriate, for example, to recommend to farmers to keep treated animals away from watercourses for a certain time following treatment in order to reduce the risk to surface waters. The time intervals should be fixed based on excretion data for the treated animal species, drug formulation, and route of application. Mitigation measures may also be necessary to reduce the risk to dung organisms. The practicability and efficacy of potential mitigation approaches remains to be established.

CONCLUSIONS

The results of the present case study clearly demonstrate that, with regard to its environmental aspects, ivermectin is a substance of high concern. The ERA of ivermectin was performed mainly according to international and European guidelines (VICH 2000, 2004; EMEA 2008), using a large number of new data on fate and effects of ivermectin and

additional results from 2-species, multispecies, semifield, and field studies obtained within the ERAPharm project. Previous ERAs for ivermectin had revealed no concern for the aquatic compartment. Effects on dung-insect populations had been considered as transient and thus not relevant. In contrast to these ERAs, the present case study—although in part preliminary—clearly demonstrates unacceptable risks (e.g., for daphnids and dung organisms) and, hence, suggests the necessity of reassessing ivermectin containing veterinary medicinal products. Furthermore, the case study indicates several gaps in the existing guidelines, which should be considered within guideline revision processes.

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