Fate and Effects of Parasiticides in the Pasture Environment

Louise Jay Pope

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The University of York

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Abstract

Parasiticides are used in the treatment of livestock animals. Following use, they may be excreted to the environment where they can impact non-target organisms. European regulations require an environmental risk assessment (ERA) for parasiticides before they are authorised for use, and guidance exists on how risks should be assessed. The methods employed are simple and conservative so it would be beneficial if approaches could be developed that more accurately assess the risks. The aim of this study was therefore to develop, through a combination of field and modelling investigations, an improved understanding of those factors and processes determining the risks of parasiticides in the pasture environment. The study focused on the avermectin compound, ivermectin (IVM). Following administration to cattle, IVM was found in manure at levels up to 1.3 mg/kg (dry weight); this is an order of magnitude greater than the No Observed Effect Concentration (NOEC) for IVM to dung flies. Once released into the field, residues persisted in dung. Small amounts of IVM were transported into soil, probably as a result of the activity of soil and dung fauna. Mesocosm studies showed that in surface waters IVM will rapidly dissipate from the water column through photodegradation and partitioning to the sediment. In sediment, the IVM is highly persistent. Matrix population modelling was used to extrapolate the results of excretion, persistence and ecotoxicity data to the wider environment. The modelling predicted only a small impact of IVM on the abundance of a fast-breeding fly, a finding supported by the results of published monitoring studies. The study has demonstrated that population modelling approaches provide a valuable tool for use in ERAs for parasiticides. The study has also highlighted a number of areas, including a rigorous consideration of analytical method performance that should be considered when assessing the fate and effects of parasiticides in the environment.

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Author's Declaration

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The work on the fate of ivermectin in aquatic mesocosms has previously been published in the journal Aquatic Toxicology*:* Sanderson, H., Laird, B., Pope, L., Brain, R., Wilson, C., Johnson, D., Bryning, G., Peregrine, A.S., Boxall, A. & Solomon, K. (2007) Assessment of the environmental fate and effects of ivermectin in aquatic mesocosms. *Aquatic Toxicology*, **85,** 229-240.

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1 Introduction

1.1 Veterinary Medicines in the Environment

Veterinary medicinal products (VMP) are used world-wide to treat animal diseases, protect animal health and to promote growth. These compounds may be released into the environment either directly or indirectly, where they could degrade; persist for a period of time; or potentially be transported to other environmental compartments. There is increasing concern that veterinary medicines may adversely affect the environment. This may occur directly by causing mortality to exposed, non-target species or indirectly, for example through a knock-on effect on organisms higher in the food chain (McCracken, 1993; Floate *et al.*, 2008).

Until recently, veterinary medicines were considered 'emerging' contaminants with assessment and modelling procedures still in early stages of development compared to other contaminant classes such as pesticides (Mackay & Mackay, 2007). While assessment methods developed for pesticides and human pharmaceuticals may be drawn upon, the routes of exposure for VM are considerably different. Pesticides are usually intentionally released in measured applications directly to crops or fields (Kolpin *et al.*, 2002). Exposure to human pharmaceuticals, household chemicals and personal care products is primarily (at least in developed countries) through sewage treatment effluent or the application of sewage sludge to land as fertiliser. In contrast, veterinary medicines may reach the environment through a number of routes (see Figure 1-1). They may be directly discharged to the environment either intentionally (e.g. aquaculture treatments in open sea cages), or unintentionally (e.g. farmyard run-off or spillage during treatment).

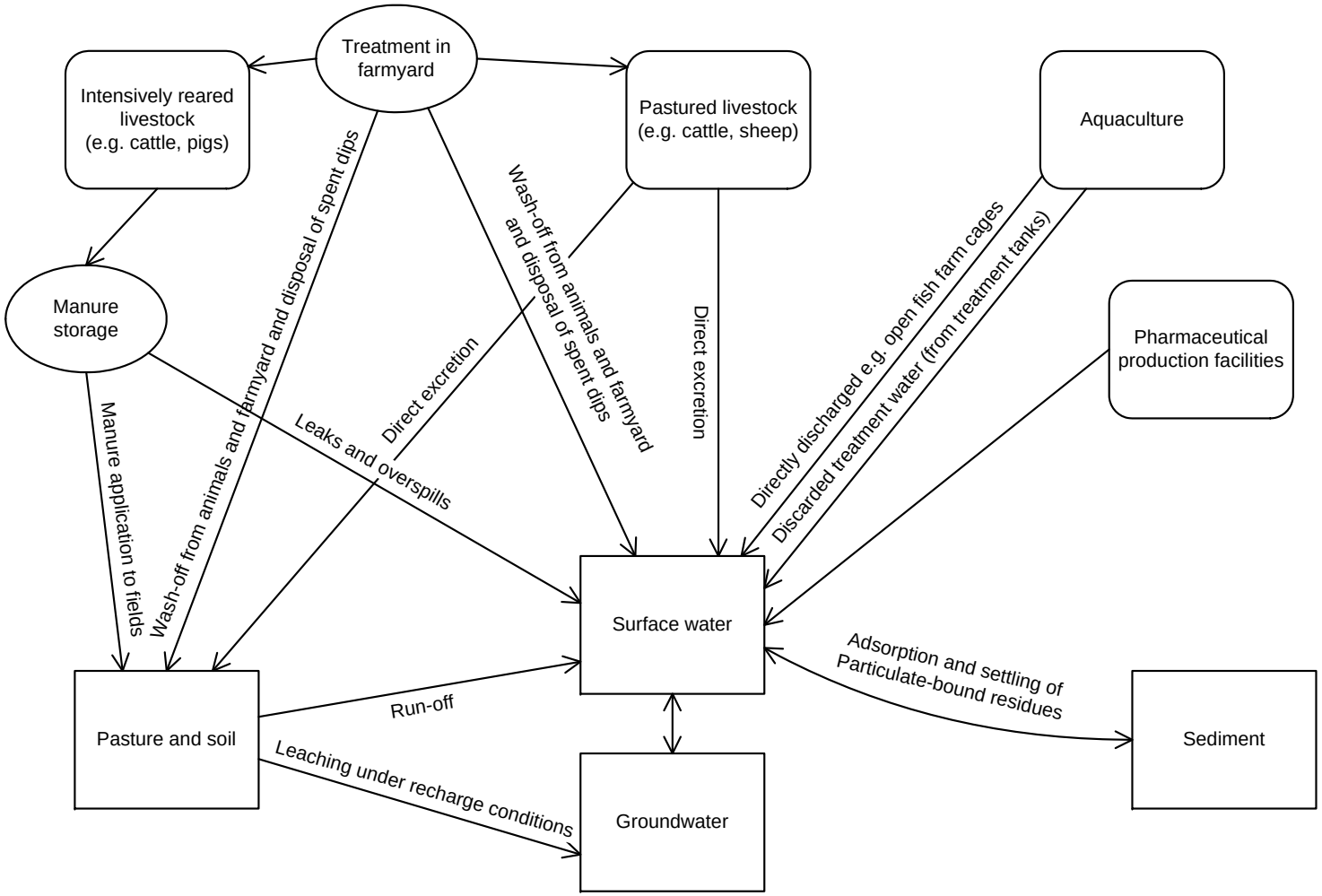


Figure 1-1 Routes of environmental exposure for veterinary medicinal products

In the terrestrial environment, exposure to veterinary medicines is most likely to occur following excretion from treated animals and therefore the medicines will be released into the terrestrial system within an organic-rich manure (faeces and/or urine) matrix which may effect the compounds' fate and bioavailability (e.g. Boxall *et al.*, 2002; Thiele-Bruhn & Aust, 2004). Other environmental compartments may also be exposed, depending on the use scenario and the properties of the compound. Residues may be transported to different compartments depending on the persistence and properties of the particular compound. For example, surface waters may be exposed indirectly by surface run-off following manure application to fields if the compound is at least moderately persistent (e.g. Burkhardt *et al.*, 2005). Concentrations within the various environmental compartments will depend on the formulation or treatment method (e.g. topical, in-feed or injection), the degree of metabolism in the target animal, treatment regime and characteristics of the VMP.

Pharmaceuticals are designed to elicit biological effects in target animals (Lissemore *et al.*, 2006). Concern has therefore been raised that veterinary medicines may also affect non-target animals once released to the environment. Undesired effects of pharmaceuticals may occur because organisms in the environment have the same receptor as that which the medicine targets in the target organism (e.g. parasiticides on dung fauna), or because the compound elicits completely different effects (e.g. endocrine disruption) than those for which it is designed (Lissemore *et al.*, 2006). In addition to the more general concerns of effects on soil microbiology; toxicity to non-target aquatic, soil and dung fauna and the possible knock-on effects on other organisms in the food chain; there are specific concerns regarding secondary poisoning, endocrine disruption and increasing antibiotic resistance.

One controversial issue is the contribution veterinary antibiotics make to increasing antibiotic resistance in the environment, where the overuse of antibiotics may lead to local bacterial populations becoming resistant. The increase in antibiotic resistance in the environment may be the result of an increase in the dominance of resistant bacteria through selective pressure (Khan

et al., 2008) or the horizontal transfer of genes coding for resistance to other bacterium species (Byrne-Bailey *et al.*, 2009; Heuer & Smalla, 2007; Stine *et al.*, 2007). The link between the increase in resistant pathogenic bacteria and the increased use of antibiotics is now generally accepted (Van Den Bogaard & Stobberingh, 2000) and there is growing evidence for a contribution from veterinary antibiotics to the distribution of resistance, the transfer of resistant bacteria from manure to soil and the horizontal transfer of resistance to other bacterium species (e.g. Byrne-Bailey *et al.*, 2009; Heuer & Smalla, 2007; Stine *et al.*, 2007).

Some veterinary medicines are thought to be endocrine disrupters: chemicals of either hormone origin or capable of inducing a hormonal response in exposed organisms. For example, steroidal hormones may be administered as growth promoters in intensively-reared livestock. Once released to the environment, these chemicals can disrupt the endocrine system of non-target aquatic animals by mimicking or antagonizing endogenous hormones (Xuan *et al.*, 2008; Wilson *et al.*, 2002; Sumpter, 2005). Although use of hormones as growth promoters is prohibited in the EU, several hormones, both endogenous (e.g. 17β-estradiol, progesterone, testosterone) and synthetic (trenbolone acetate, zeranol), are used as growth promoters in the US, Australia and other countries (Ingerslev *et al.*, 2003; Khan *et al.*, 2008).

One of the more widely-reported incidents of an environmental impact of veterinary medicines is the drastic decline in vulture populations in Pakistan and India caused by secondary poisoning from diclofenac (Green *et al.*, 2004; Oaks *et al.*, 2004). Populations of three vulture species had declined by over 95% within 10 years, leading the IUCN to list the three species as critically endangered (Green *et al.*, 2004). Eventually, Oakes *et al.*, proposed that these population declines were linked to secondary poisoning from diclofenac, a non-steroidal, anti-inflammatory drug used in cattle (Oaks *et al.*, 2004). Vultures were found to be exposed to lethal doses of diclofenac through feeding on the carcasses of treated cattle (Oaks *et al.*, 2004). Subsequent investigations have shown similar associations from dead and dying vultures in Nepal and India (Green *et al.*, 2004).

It is clear from these studies that veterinary medicines will be released to the environment and as a result there is concern over the impacts of these substances in biota. As the main focus of this PhD is on terrestrial systems, in the following sections, a more detailed discussion of the use, inputs, fate and ecotoxicity of veterinary medicines in the terrestrial environment is provided.

1.2 Use of Veterinary Medicinal Products in the Terrestrial Environment

Veterinary medicinal products are used for a variety of purposes in animal health as: anaesthetics, antacids, anthelmintics, antihistamines, anti-infectives, steroidal and non-steroidal anti-inflammatories, antibacterials, antimicrobials, antiseptics, astringents, bronchodilators, diuretics, emetics, growth promoters, sedatives, tranquilizers and for the synchronisation of estrus (Sarmah *et al.*, 2006). These medicines may be administered as an injection (subcutaneous or intramuscular), topically (pour-on, spot-on or dipping), orally (oral drench, or via feed or water), or in a bolus which is designed to release the VM over a sustained period of time. The VMPs most likely to reach the terrestrial environment and cause impacts there are those used to treat livestock and poultry, whether reared outdoors or more intensively indoor-reared. These include antibiotics, parasiticides, antifungals and compounds used for hormonal control. Table 1-1 summarises the main chemical classes of VMPs used to treat livestock and poultry in the UK.

Antibiotics can be used to treat bacterial infections or given at low, sub-therapeutic doses as growth-promoters for intensively-reared livestock. It is the extensive use of antibiotics given to animals in the absence of disease that has attracted criticism in the light of increased resistance in the environment. Concerns that use of veterinary antibiotics may reduce the effectiveness of antibiotics used in human health has caused the World Health Organisation to discourage the use of veterinary antibiotics that are similar in structure (belonging to the same class of compounds) as those currently used in human medicines. The EU has now largely banned the use of antibiotics as growth promoters. The only antibiotics still registered for use as growth promoters (or

as feed additives) in the EU are avilamycin, monensin, salinomycin and bambermycin (Sarmah *et al.*, 2006). The situation is quite different in USA however. A report published in 2001 by the Union of Concerned Scientists estimated that 70% of the antibiotics produced in USA are used for non-therapeutic purposes, equating to approximately 11.2 million kilograms per year (Mellon *et al.*, 2001).

Table 1-1 Classes of compounds used as livestock VMPs in the UK, NOAH (2008)

Function	Class	Compound	Primary Use
Antibiotics	Aminoglycosides	Apramycin	Pigs, cattle, poultry
		Kanamycin	Cattle
		Neomycin	Cattle
		Spectinomycin	Pigs, cattle, poultry, sheep
	β- lactams: penicillins	Amoxicillin	Cattle, sheep, pigs
		Ampicillin	Cattle, sheep, pigs
		Cloxacillin	Cattle
	Cephalosporines	Ceftiofur	Cattle, pigs
		Cefquinome	Cattle, pigs, horses
	Fluorochinolones	Enrofloxacin	Cattle, pigs, poultry
		Marbofloxacin	Cattle, pigs
	Lincosamides	Lincomycin	Pigs, cattle
	Macrolides	Erythromycin	Poultry
		Tylosin	Cattle, poultry, pigs
	Organophosphate	Diazinon	Sheep
	Sulphonamides	Sulphadimethoxine	Cattle, pigs
		Sulphadiazine	Cattle, horses, pigs, poultry
		Sulphadoxine	Cattle, horses
	Trimethoprim	In combination with sulphonamides	
	Tetracyclines	Chlortetracycline	Cattle, pigs, poultry
		Oxytetracycline	Cattle, sheep, pigs
		Tetracycline	Poultry, pigs
	Tetrahydropyrimidine	Morantel tartrate	Cattle
Parasiticides	Macrocyclic lactones	Ivermectin	Cattle, sheep, pigs, horses
		Eprinomectin	Cattle
		Doramectin	Cattle, sheep, pigs
		Moxidectin	Cattle, sheep, horses
	Benzimidazoles	Albendazole	Cattle, sheep
		Fenbendazole	Cattle, sheep, pigs, horses
		Oxfendazole	Cattle, sheep
		Triclabendazole	Sheep, cattle
	Imidazothiazole	Levamisole	Cattle, sheep
	Synthetic pyrethroids	Cypermethrin	Sheep, cattle
		Deltamethrin	Cattle, sheep

The other main group of VMPs is the parasiticides, which will be the main focus of this thesis. Parasiticides can be classified as endo-parasiticides, ecto-

parasiticides or endectocides. Endoparasiticides are used to control internal parasites and include anthelmintics (wormers), flukicides, tapewormers, antiprotozoals and coccidiostats. Ecto-parasiticides are used to control external parasites. Endectocides simultaneously control both internal and external parasites. A recent survey of English dairy and beef farms found the most frequently used parasiticide was ivermectin, followed by oxfendazole, eprinomectin, doramectin, fenbendazole, morantel, permethrin and moxidectin (Boxall *et al.*, 2007).

1.3 Inputs to the Terrestrial Environment

The main route through which veterinary medicines reach the terrestrial environment is through the excretion of residues from treated animals. The amount of the VMP reaching or entering the environment is determined by the compound's excretion profile for the target animal. Excreted residues, either as the parent (treatment) compound or metabolites (or a mixture of both), may then reach the soil via the fertilisation of land with stored manure, excretion from pastured livestock or farm-yard run-off (Boxall *et al.*, 2004).

1.3.1 Pastured Livestock

Excretion

After animals have been treated, the active ingredient of a VMP may be excreted as the parent compound, metabolites, or a mixture of both. VMPs can be poorly metabolised with up to 75% of the applied dose excreted as the parent compound (Elmund *et al.*, 1971). This can result in high concentrations of the medicine being detected in manure. The route of excretion will also vary, with some substances being excreted via urine and some via faeces, which may be important in determining the environmental impact of a substance. Figure 1-2 shows the major excretion route for a range of parasiticides used in the UK. The avermectins as a group, tend to be excreted in the faeces (Chiu *et al.*, 1990; Hennessy *et al.*, 2000). The excretion route of the benzimidazoles as a group is

more variable, with albendazole residues largely excreted in the urine (~60%) and oxfendazole in the faeces (~80%) (Hennessy *et al.*, 1993a).

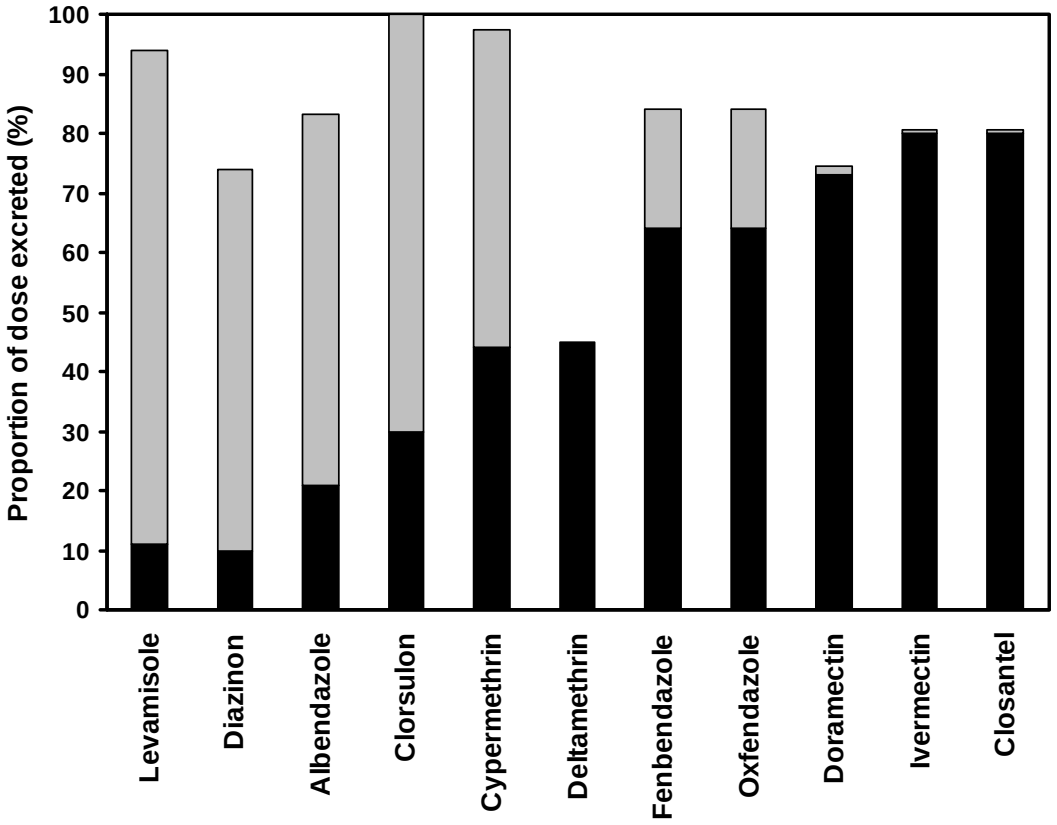


Figure 1-2 Percentage of applied dose excreted in dung (black) and urine (grey) (Paulson & Feil, 1996; Inchem, 1970; Hennessy *et al.*, 1993a; Croucher *et al.*, 1985; Juliet *et al.*, 2001; Hennessy *et al.*, 1993b; Hennessy *et al.*, 2000; Chiu *et al.*, 1990; Inchem, 2006).

Veterinary medicines which are more extensively metabolised tend to be ultimately excreted in the urine, as the metabolic processes in the animal generally result in the formation of more polar and water soluble metabolites. For example, while approximately 80% of the dose of levamisole applied to cattle was excreted in the urine, the parent compound was undetected (Paulson & Feil, 1996) and diazinon, another extensively metabolised VMP, is largely excreted in the urine (65%) with less than 1% detected as the parent compound (Inchem, 1970). Residues of veterinary metabolites undergoing less or minimal metabolism are more likely to be excreted in the faeces. For example, large proportions of radio-labelled ivermectin and eprinomectin were detected in the faeces as parent compounds, at 45 and 86% respectively (Halley *et al.*, 1989c; Inchem, 1998). Therefore, for pastured animals at least, parent compounds,

which are usually *but not always* more active than their metabolites, will remain associated with the faeces. As the metabolites excreted in the urine are more soluble, they are more rapidly and easily dissipated.

The method of application can also be a key factor in determining a compound's excretion profile. Common application methods are injection (subcutaneous or intramuscular), topical (pour-on, spot-on or dipping), oral (oral drench, or via feed or water), or a bolus. The majority of available data on the effect of different application methods on a compound's elimination profile concern ivermectin and will be discussed in more detail in Chapter 3. In general, for the avermectins, pour-on treatments tend to result in higher and more variable concentrations in manure compared to injectable treatments and excretion is more rapid following oral treatment.

Wash-off from Farm-yards and Treated Animals

For topically applied VMPs, wash-off from both recently treated animals and the farm-yard where treatment occurred can be a route to the environment (e.g. Ramwell *et al.*, 2007). Several synthetic pyrethroids and avermectins are available as pour-on treatments and since they are applied externally they have the potential to be washed off the animal if rainfall occurs soon after treatment. However, it is the sheep-dip treatments in which the whole animal is submerged that cause more concern. During sheep-dipping the whole animal is submerged and then held in drain pens, which drain back into the treatment bath, for either 10 minutes or until the animals have stopped dripping, after which the sheep may be kept in the farm-yard for a period of time before release back to pasture.

Two recent studies published by DEFRA investigated the potential for cypermethrin, a synthetic pyrethroid used in the UK in sheep-dips (although currently under review), to be washed off the fleeces of dipped sheep and the hard-standing in the farm-yard during normal dipping practice (Sinclair *et al.*, 2007; Ramwell *et al.*, 2007). In one study, the sheep were dipped, held in the drip drain for 10 minutes and transferred to holding pens in the farmyard to dry off

for different periods of time before being sent through a metal trough of water simulating a stream (Ramwell *et al.*, 2007). After one hour of drying a mean of 0.9 mg (per sheep) of cypermethrin was found to transfer from the animal to the water and even after two days of drying time nearly 0.2 mg per sheep was transferred to the water. As well the possibility of residues being washed off treated animals, Sinclair *et al.,* (2007) found that in 87% of the farms in their survey, the sheep had access to streams in their normal pastures.

There is also evidence for residues on the hard-standing in farmyards used for sheep dipping to be washed off with rain. Ramwell *et al.,* (2007) measured a mean of level 2.6 mg/m^2 of cypermethrin on the concrete of the holding pens, an area separate from the drain pens. Sinclair *et al.,* (2007) monitored cypermethrin concentrations in the run-off from a farm-yard used for sheep dipping following irrigation and natural rainfall. Peaks of up to 30 µg/L were measured during periods of heavy rainfall followed by continuous, smaller releases in subsequent rainfalls, the majority of which (~90%) was associated with particulate matter.

1.3.2 Intensively Reared Livestock

The amendment of agricultural soil with manure is a potential non-point source input of VMPs (Lissemore *et al.*, 2006). In a recent review of chemical contamination in feedlot wastes, Khan *et al.*, (2008) reported steroidal hormones, antibiotics, parasiticides, mycotoxins, heavy metals and dioxins as the compound groups of particular environmental and human-health concern. The excrement (faeces and urine) of intensively-reared livestock such as pigs and cattle are collected and stored together with remnants of food and bedding as slurry for anything up to 1 year before application to land. This can be a major route of environmental exposure to VMs. For instance, in the US concentrated animal feeding operations (CAFOs) produce up to 133 million tons (dry weight) of manure per year (Burkholder *et al.*, 2007).

In the US and Canada, stored manure or slurry is generally kept in anaerobic lagoons - large outdoor basins (Kuchta & Cessna, 2009). The slurry is only in

contact with air and sunlight at the surface, limiting the extent of photo-degradation and maintaining anaerobic conditions at depth (Schlusener *et al.*, 2006). In Europe, slurry is usually held in anaerobic tanks and may be a mixture of pig and cattle waste (Schlusener *et al.*, 2006 & personal observation).

As manure is produced throughout the year and the slurry tanks are emptied periodically, e.g. in Europe, February to April for cereals and September for rapeseed (Lopez-Ridaura *et al.*, 2009), storage time may be anything from 5 months to a year. Conditions such as temperature, redox, organic matter content and pH will vary according to storage methods and will affect the degradation rates of VM during storage, altering concentrations before application to land. If aeration, water content and carbon to nitrogen rations are managed, stored manure can be composted, accelerating the rate of VM degradation (Khan *et al.*, 2008).

Manure applied to land may contain a mixture of parent compounds, metabolites (formed in the animal) and degradation products (formed in storage). There is also potential for residues excreted as conjugates to revert back to the parent compound in manure storage, e.g. sulfonamides (Boxall *et al.*, 2002). Depending on the compound, these VM residues then have the potential to contaminate the soil and reach surface waters via run-off and groundwater by leaching. Further exposure routes include leaks from poorly constructed lagoons and overflow following intense rainfall (Burkholder *et al.*, 2007).

Table 1-2 summarises the concentrations detected in manure from intensively-reared animals. In freshly excreted manure the tetracyclines oxytetracycline and chlortetracycline can be present in concentrations of up to 872 mg/kg (cattle manure) and 108 mg/L in (pig slurry) and tylosin at concentrations up to 115 mg/kg (cattle manure) (Winckler & Grafe, 2001; De Liguoro *et al.*, 2003). High concentrations of the fluoroquinolones, enrofloxacin and its metabolite ciprofloxacin have also been reported in fresh pig excreta, of up to 24 and 48 mg/L respectively (Zhou *et al.*, 2008). Concentrations in stored slurry might be expected to be lower due to the dilution of manure from untreated animals and water from cleaning the animal houses. However, high concentrations (>1 ppm)

have been measured in stored slurry for tetracycline, chlortetracycline and the sulphonamides sulfathiazole and sulfamethazine (Thiele-Bruhn & Aust, 2004; Winckler & Grafe, 2001; Campagnolo *et al.*, 2002; Haller *et al.*, 2002). Concentrations in stored manure will depend on the dose rate of the compound, the proportion of animals treated (high for antibiotics used in feed as growth promoters), storage practices (e.g. if soiled bedding collected or separated) and the persistence of the compound, in which case the manure storage time becomes more important.

Table 1-2 Concentrations of VMP measured in manure and slurry from intensively reared animals, * = unknown if concentration is in terms of wet or dry weight, likely to be wet weight

Class	Substance	Matrix	Peak Concentrations (µg/kg ww unless otherwise stated)	Reference
Fluoroquinolone	Enrofloxacin	Pig manure (freshly excreted) Pig urine (freshly excreted)	24,680* 22,740 µg/L	Zhou *et al.*, (2008)
		Pig slurry lagoons	5 µg/L	Campagnolo *et al.*, (2002)
	Ciprofloxacin (enrofloxacin metabolite)	Pig manure (freshly excreted) Pig urine (freshly excreted)	30,980* 48,040 µg/L	Zhou *et al.*, (2008)
Ionophores	Salinomycin	Pig slurry (stored for several months)	11*	Schlusener *et al.*, (2003)
Lincosamides	Lincomycin	Pig slurry	9,870 µg/L	Kuchta & Cessna (2009)
		Pig slurry lagoon	240 µg/L	Campagnolo *et al.*, (2002)
Macrolides	Erythromycin	Pig slurry lagoon	275 µg/L	Campagnolo *et al.*, (2002)
	Tylosin	Cattle faeces (freshly excreted)	115,500*	De Liguoro *et al.*, (2003)
	Tiamulin	Pig slurry (stored for several months)	43*	Schlusener *et al.*, (2003)
Sulphonamides	Sulfadiazine	Chicken and Turkey manure	91,000*	Martinez-Carballo *et al.*, (2007)
	Sulfathiazole	Stored pig slurry	12,400 ww	Haller *et al.*,(2002)
	Sulfamethazine	Pig slurry	11,000 µg/L	Burkhardt *et al.*, (2005)
		Pig manure	20,000*	Martinez-Carballo *et al.*, (2007)
		Stored pig slurry	8,700 ww	Haller *et al.*,(2002)
		Stored cattle slurry	3,200 ww	Haller *et al.*,(2002)

Table 1-2 continued Concentrations of VMP measured in manure and slurry from intensively reared animals, * = unknown if concentration is in terms of wet or dry weight, likely to be wet weight

Class	Substance	Matrix	Peak Concentrations (µg/kg ww unless otherwise stated)	Reference
Sulphonamides	Sulfamethazine	Pig slurry lagoons	400 µg/L	Campagnolo *et al.*, (2002)
	Acetyl-sufamethazine (sulfamethazine metabolite)	Stored pig slurry	2,600 ww	Haller *et al.*,(2002)
	Sulfadimethoxine	Swine lagoon	2.5 µg/L	Campagnolo *et al.*, (2002)
Tetracyclines	Chlortetracycline	Pig manure	46,000*	Martinez-Carballo *et al.*, (2007)
		Pig slurry (freshly excreted)	108,000 µg/L	Winckler & Grafe (2001)
		Pig slurry lagoons	1000 µg/L	Campagnolo *et al.*, (2002)
	Oxytetracycline Tetracycline	Liquid manure	100*	Hamscher *et al.*, (2002)
		Pig manure	29,000	Martinez-Carballo *et al.*, (2007)
		Cattle faeces (freshly excreted)	871,700	De Liguoro *et al.*, (2003)
		Stored pig slurry	66,000 (mean 11.6) µg/L	Winckler & Grafe (2001)
		Pig manure	23,000	Martinez-Carballo *et al.*, (2007)
		Liquid manure	4,000*	Hamscher *et al.*, (2002)
	Spectinomycin	Pig slurry	686 µg/L	Kuchta & Cessna (2009)
	Trimethoprim	Pig lagoon	2.5 µg/L	Campagnolo *et al.*, (2002)

1.4 Fate in the Terrestrial Environment

To assess the concentrations of VMP residues to which organisms may be exposed and over what timescale the exposure occurs, it is necessary to consider the fate of the VM once it enters the environment. Factors such as partitioning to organic matter or sediment may reduce a compound's bioavailability and are a key factor in determining its movement between manure, soil, surface waters and ground water (Boxall *et al.*, 2004). The compound may persist for some time in the environment or be subjected to biodegradation, photo-degradation or hydrolysis.

1.4.1 Occurrence in Soil

Soil may be exposed to VMP residues following amendment with manure from treated animals or contact with residues directly excreted from pastured animals. Soil exposure from the excretion of pastured livestock is likely to be patchy in nature with exposure restricted to movement across the manure–soil interface. This interface is obviously much larger for liquid manure spread onto land. We have also seen that liquid manure can contain very high levels of VMP, especially for the tetracycline and macrolide antibiotics.

VMP may enter the soil by a variety of mechanisms. More soluble and less tightly sorbed residues may be leached from the manure into the soil; particulate-bound residues may be physically incorporated into the soil by ploughing or transported by earthworm and other soil invertebrate activity or via preferential flow through small channels in the soil. Some of these mechanisms will be explored in more detail in Section 1.4.3.

Despite reports of high levels of VMPs in liquid manure and in pastured livestock excreta, the concentrations in soil and their fate are less understood. Table 1-3 summarises the concentrations of a limited number of VMPs measured in soils either following manure application or in the soils of pastured livestock.

Table 1-3 Peak concentrations of VMP measured in soils, * = unknown if concentration is in terms of wet or dry weight, likely to be wet weight

Class	Substance	Soil Description	Concentration (µg/kg)	Reference
Avermectins	Abamectin	Pasture of treated sheep	1.4 dw	Erzen *et al.,* (2005)
Fluoroquinolone	Enrofloxacin	20 cm 40 cm	204* 18*	Uslu *et al.,* (2008)
	Ciprofloxacin (enrofloxacin metabolite)	20 cm 40 cm	53* nd	Uslu *et al.,* (2008)
Macrolides	Tylosin	0- 60 cm	<10 (LOD)*	De Liguoro *et al.,* (2003)
Tetracyclines	Chlortetracycline	0- 30 cm	7.3 dw	Hamscher *et al.,* (2002)
	Oxytetracycline	0-5 cm 5-10 cm	270.05* (22 days after manure) 0.81* (70 days after manure)	Aga *et al.,* (2005)
		0-30 cm 60 cm	Detected but below LOQ (10) < 5* (LOD)	De Liguoro *et al.,* (2003)
	Tetracycline	0-10 cm 10-20 cm 20-30 cm	86.2 dw 198.7 dw 171.7 dw	Hamscher *et al.,* (2002)
		0-5 cm 5-10 cm	11.29* (22 days after manure) 0.6* (70 days after manure)	Aga *et al.,* (2005)

Relatively high concentrations of the tetracyclines of up to 270 µg/kg have been detected in manure-amended soils (Hamscher *et al.*, 2002; Aga *et al.*, 2005; De Liguoro *et al.*, 2003). In addition, as time after treatment increased, tetracycline residues were also detected in subsurface soil layers. In contrast, little to no tylosin was detected following manure application with confirmed tylosin residues present, indicating relatively rapid dissipation rates in the soil (De Liguoro *et al.*, 2003).

1.4.2 Fate Processes in Soil

Once a veterinary medicine has entered the environment, residues may leach into the soil, ultimately exposing groundwater, or be transported to surface waters or sediments via run-off in either the aqueous or particulate phases (Boxall *et al.*, 2004). The extent to which a compound is transported by these routes will be strongly influenced by the compound's sorption behaviour and the nature (e.g. pH) of the matrix it is in (e.g. soil, cattle manure or pig slurry). For neutral, hydrophobic compounds, sorption may be adequately expressed by its K_{oc}, the organic carbon partitioning coefficient. A classification scheme for pesticides describes compounds with a K_{oc} of less than 15 to be highly mobile and those with a K_{oc} higher than 4,000 to be effectively non-mobile (Hollis, 1991).

Sorption of pharmaceuticals is not always predictable by their hydrophobicity alone. The speciation of these often large, complex molecules may also be affected by the pH of the matrix (Tolls, 2001). In these cases, other sorption mechanisms may be more important such as cation exchange, cation bridging, surface complexation and hydrogen bonding (Tolls, 2001). The addition of manure to soil is known to increase the soil pH as well as increasing microbial activity and organic carbon content (Kreuzig *et al.*, 2007; Boxall *et al.*, 2002) affecting the sorption behaviour of certain compounds. Although the exact extent of partitioning between solid matrices (e.g. manure or soil) and aqueous phase is not always perfectly described by its $K_{oc,}$ this is still a useful indication of the aqueous mobility in the presence of soil and manure for many VMPs.

The stability or persistence of VMs contributes to their distribution in the environment. A smaller proportion of a VM will be available for transport if it is rapidly degraded in dung or slurry. The most frequently detected substances in a monitoring survey performed by Lissmore *et al.,* (2006) were those most resistant to microbial breakdown and hydrolysis. Losses due to volatilisation will be negligible, as most VMs have low vapour pressure and Henry's law constants. Veterinary medicine residues may undergo biodegradation in soil or manure, hydrolysis and direct and indirect photolysis in water. The extent of degradation will depend on the compound characteristics (e.g. concentration if the compound inhibits biodegradation) and local conditions such as temperature, moisture, whether anaerobic or aerobic and soil type (Wang *et al.*, 2006; Kuhne *et al.*, 2000).

Table 1-4 illustrates the range of persistence and sorption capabilities for a range of VMPs in soil and manure. A large proportion of VMP compounds have been classified as very persistent and slightly- to non-mobile, in particular, the benzimidazoles (albendazole, fenbendazole and oxfendazole) and the macrocyclic lactones (abamectin, doramectin, eprinomectin, ivermectin and moxidectin), all of which are used as parasiticides. There is some indication that persistence and mobility of these compounds are affected by the matrix and the conditions in which they exist. Ivermectin has been reported to degrade more rapidly in certain soils and under warmer conditions as opposed to in manure or in cooler conditions (Krogh *et al.*, 2009; Halley *et al.*, 1989b). Fenbendazole was found to degrade more rapidly in clay soils in the presence of manure than in soil without manure, with degradation half lives of 9 and 54 days respectively and both fenbendazole and flubendazole were found to degrade more slowly in sand soils compared to clay soils (Kreuzig *et al.*, 2007). The relatively high K_{oc} reported for all these compounds indicates a high affinity for organic matter. These compounds are likely to remain associated with soil and/or manure and therefore are not expected to leach into the soil column. This was confirmed for flubendazole and fenbendazole which were not detected in the subsoil of irrigated, manured test plots for up to 330 days (Kreuzig *et al.*, 2007). The low mobility of ivermectin and abamectin was confirmed using radio-labelled

compounds in soil column studies where 39-45% (ivermectin) and 92-79% (abamectin) of the radioactivity was retained in the top 5 cm respectively and nil to <10% was detected in the leachate (Halley *et al.*, 1989a; Gruber *et al.*, 1990). If these compounds reach aquatic systems (e.g. via treated livestock excreting over water bodies), residues are unlikely to partition into the water and will either remain associated with the manure or sorb to the sediment. Given the slow degradation rates for these chemicals, one concern may be the build up of residues in the soil surface following repeated use by treated livestock.

Of the VMs presented in Table 1-4, the antibiotics ceftiofur, florfenicol, metronidazole, olaquindox and clorsulon were the most mobile compounds and therefore the most susceptible to transport in the aqueous phase, either into the soil column or via surface run-off. The mobility of these compounds may be mitigated by their fairly rapid degradation rates, although rates may change in presence of manure (e.g. clorsulon appears to be more persistent in manure, Merck (1985)).

The fate of the macrolide tylosin appears to vary between different soils and between soils and manure. In general, although tylosin is relatively soluble, and can be relatively mobile in liquid manure (Loke *et al.*, 2002), it does tend to sorb to soil with K_{oc}s between 500 to 95,000 (Sassman *et al.*, 2007; Rabolle & Spliid, 2000; Carlson & Mabury, 2006). This low potential to leach into the soil column was confirmed in a soil column test where 60-80% of the tylosin remained in the top 5cm of soil and was not detected in the leachate (Rabolle & Spliid, 2000). The fate of other macrolides is less comprehensively described. Tiamulin has been found to be highly persistent in manure storage with no change in concentration observed in 180 days, but less persistent in soils, with a half-life of 16 days (Schlusener & Bester, 2006; Schlusener *et al.*, 2006). Salinomycin and erythromycin are also less persistent in soils than manure (Schlusener *et al.*, 2006).

Table 1-4 Mobility and persistence of a range of active ingredients used in veterinary medicines using Hollis's (1991) classification system, a less comprehensive version of this table was previously published in Pope *et al.*, (2009)

Mobility	Non-persistent (DT_{50} < 5 days)	Slightly persistent (DT_{50} 5–21 days)	Moderately persistent (DT_{50} 22–60 days)	Very persistent (DT_{50} > 60 days)	Unknown persistence
Mobile (K_{oc} 15-74)		Ceftiofur (soil)[21] Clorsulon (soil)[20] Florfenicol (soil)[21] Metronidazole (soil)[10, 11]	Clorsulon (manure)[20] Fenbendazole (silt loam)[31]		Olaquindox (pig manure)[30]
Moderately mobile (K_{oc} 75-499)	Sulfadimethoxine[17,39]	Diazinon (soil)[19] Erythromycin (loam, sandy loam)[14,33] Olaquindox[10, 11] Tylosin A (pig manure)[30]		Diclazuril (silty clay loam)[24]	Danofloxacin (chicken manure)[28] Oxytetracycline (pig manure) [30] Sulfapyridine[17] Sulfathiazole[29] Sulfamethazine[29]
Slightly mobile (K_{oc} 500-4000)	Tylosin (soil + manure)[11,13,16]	Fenbendazole (clay soil + manure)[8] Tylosin (soil) [11,13,16]	Fenbendazole (clay soil, lab & field)[8]	Diclazuril (sandy loam and silt loam) [24] Oxfendazole (soil)[25]	Danofloxacin (cattle manure)[28] Efrotomycin (loam, silt loam)[22]
Non-mobile (K_{oc} >4,000)		Cypermethrin (soil)[23,27] Ivermectin (Madrid soil, 'summer'soil)[7, 35] Erythromycin (silty clay loam)[14,33] Tylosin (soil)[11,14]	Avermectin B1a (soil)[1,2] Ivermectin (Tastrup soil)[7, 35] Deltamethrin (soil)[38] Erythromycin (manure storage)[15]	Albendazole[26] Danofloxacin (soil)[28] Doramectin (soils, manure)[3, 4] Efrotomycin (sandy loam, clay loam) [22, 32] Eprinomectin (soil)[6] Flubendazole (clay soil, clay soil+ manure, lab & field)[8] Ivermectin (york soil, 'winter' soil, artificial soil)[7, 35] Moxidectin[34] Oxytetracycline (soil)[11,29]	Emamectin benzoate (soil)[5] Enrofloxacin (soil)[9,29]
Unknown K_{oc}	Monensin (K_{oc} in soils 126 – 6300)[12,13] Salinomycin (soil)[14] Sulfachloropyridazine (soil)[18]	Salinomycin (manure storage)[15] Tiamulin (soil) [14]	Bacitracin[36] Oleandomycin (soil)[14]	Roxithromycin (soil, manure)[14,15] Sulfachloropyridazine (slurry)[18] Tiamulin (manure storage)[15] Tetracycline (manure storage)[37]	

[1]Gruber *et al.,* (1990), [2]Bull *et al.,* (1984), [3]Taylor (1999), [4]Pfizer, (1996), [5]Mushtaq *et al.,* (1996), [6]Merck (1996), [7]Halley *et al.,* (1989a), [8]Kreuzig *et al.,* (2007), [9]Norwara *et al.,* (1997), [10]Ingerslev & Halling-Sorensen (2001), [11]Rabolle & Spliid (2000), [12]Sassman & Lee (2007), [13]Carlson & Mabury (2006), [14]Schlusener & Bester (2006), [15]Schlusener *et al.*,(2006), [16]Kolz *et al.,* (2005), [17]Thiele-Bruhn *et al.,* (2004), [18]Kay *et al.,* (2004), [19]Sarmah *et al.,* (2009), [20]Merck (1985), [21]Schering (1996), [21]Gilbertson *et al.,* (1990), [22]Yeager & Halley (1990), [23]Xie & Zhou (2008), [24]Mallickrodt (1996), [25]Syntex animal health Inc (1990), [26]SmithKline (1989), [27]Hartnik *et al.,* (2008), [28]Pfizer (2002), [29]Tolls (2001), [30]Loke *et al.,* (2002), [31]Thiele-Bruhn (2003), [32]Merck (1986), [33]Sanofi (1987), [34]Fort Dodge (1997), [35]Krogh *et al.,* (2009), [36]Gavalchin & Katz (1994), [37]Winckler & Grafe (2001), [38]Tomlin (2000), [39]Wang *et al.,* (2006)

(Schlusener & Bester, 2006). The sulfonamides are classified as moderately mobile in Table 1-4, with K_{oc} ranging from 37 to 300 reported in soils (Boxall *et al.*, 2002; Thiele-Bruhn & Aust, 2004; Thiele-Bruhn *et al.*, 2004). Sulfonamide sorption has been shown to be pH-dependent, and therefore their mobility may be influenced by the increase in soil pH following manure addition (Thiele-Bruhn & Aust, 2004; Boxall *et al.*, 2002).

The sulfonamides appear to be relatively persistent in anaerobic slurry storage. Langhammer reported degradation of only up to 50% of sulfadimidine and sulfathiazole residues in pig slurry after 5 weeks storage (cited in Thiele-Bruhn & Aust, 2004)). Wang *et al.,* (2006) found sulfadimethoxine residues to degrade faster in pig slurry in warmer and wetter conditions. Sulfadimethoxine degradation was also found to decline with increasing concentration, indicating the compound was inhibiting the microorganisms responsible for the biodegradation.

The synthetic pyrethroids cypermethrin and deltamethrin have a history of use as pesticides and therefore most information available on their fate in the environment relates to their fate in soils rather than soil/manure mixtures. High K_{oc} in the range of 460,000 to 16,300,000 (Hartnik *et al.*, 2008) indicate these compounds will adsorb very strongly to manure and soil and limit their mobility in the environment. They are only slightly-to-moderately persistent in soils, but no data is currently available for their persistence in manure.

The tetracyclines have relatively high water solubilities of 600, 1000, 1700 mg/L for chlortetracycline, oxytetracycline and tetracycline respectively (Sarmah *et al.*, 2006). However, these compounds do actually adsorb strongly to soil (Carlson & Mabury, 2006), with reported K_d (soil-water adsorption coefficient) of 417 to 1026 and K_{oc} of 28,000 to 93,000 for oxytetracycline in soil (Rabolle & Spliid, 2000). The sorption of tetracyclines is likely to be strongly influenced by pH and ionic binding to Mg^{2+}, Ca^{2+} and other charged ions in the matrix (Khan *et al.*, 2008). The strong sorption of the tetracyclines limits their leaching potential. This is supported by a soil column study performed by Rabolle and Spliid

(2000), where oxytetracycline was applied to sandy loam soil; no oxytetracyline was detected in the leachate.

1.4.3 Transport

Leaching

The more soluble and mobile VMs (those that adsorb poorly to soils and organic matter, as described above) may infiltrate the soil and may eventually reach groundwater. The groundwater monitoring study conducted by Barnes *et al.*, (2008) detected several antibiotics, used in veterinary medicines, in groundwater suspected to be contaminated by animal and human wastewater. However, since most of these compounds are also used in human medicine it is difficult to attribute their occurrence to veterinary sources and not to sewage treatment plants or soils amended with sewage sludge.

Transport of Particulate-bound Residues

Less soluble VMPs and those with a high affinity for soils and organic matter still have the potential to be transported in the environment by the physical transport of particulate-bound residues. These particulate-bound compounds can be incorporated into the soil via preferential flow (e.g. worm channels or fissures) or by the action of soil and dung fauna. For example, avermectin residues have been detected in the soil. A study of abamectin and doramectin degradation rates in a sheep-grazed pasture reported concentrations of up to 1.4 µg/kg (dry weight) in pasture soils (Erzen *et al.*, 2005). Given the avermectins' low solubility and high affinity for soil and manure, it is unlikely that the residues moved into the soil associated with the aqueous phase. It is more likely that the residues were incorporated by soil and dung organisms or washed into the soil as degraded dung particulates in macropores.

Surface Run-off

Whether associated with the aqueous or particulate phase (e.g. bound to soil colloids), VMs may be transported to streams and rivers via run-off or field drainage when rainfall exceeds the infiltration rate of soil. For example, a survey of wells, field tiles, streams and springs in-and-around pig and poultry farms detected lincomycin, sulfamethazine, sulfadimethoxine, trimethoprim and sarafloxacin at concentrations up to 4 µg/L (Campagnolo *et al.*, 2002). On reaching surface waters, residues may remain in the aqueous phase or partition into the sediment (and partition back into the aqueous phase from the sediment). The extent of transport in the environment may depend on the matrix properties (e.g. pH), hydraulic loading characteristics and crop management practices for soil amended with manure e.g. degree of incorporation into soil or cultivation (Burkholder *et al.*, 2007).

Food Chain

Veterinary medicine residues may also be transported within the environment through contamination of the food chain, resulting in the exposure of organisms higher up in the food chain. The exposure of vultures to toxic diclofenac residues via the abandoned carcasses of recently-treated cattle on the Indian subcontinent and in Africa is one example (Oaks *et al.*, 2004; Green *et al.*, 2004; Naidoo *et al.*, 2009), mentioned in Section 1.1.

Veterinary medicines can also be taken up by plants grown in soil amended with manure from treated animals, a potentially important exposure route to other organisms (Kong *et al.*, 2007). In a plant-uptake study, florfenicol, levamisole and trimethoprim were detected in lettuces exposed to soil spiked with VMs, with concentrations of 15, 170 and 6 µg/L respectively (Boxall *et al.*, 2006b). The same study also found diazinon, enrofloxacin, florfenicol and trimethoprim to be taken up by carrots, measuring concentrations of 13, 2.8, 5 and 53 µg/kg respectively (Boxall *et al.*, 2006b). When Kong *et al.,* (2007) investigated the uptake of oxytetracycline into alfalfa; they found uptake was slightly pH

dependent, indicating that soil pH could be an important factor in determining oxytetracycline bioavailability to plants.

Furthermore, a study conducted by Kinney *et al.,* (2008) found the VMs trimethoprim and diazinon to be present in earthworms (at concentrations of up to 127 and 61 µg/kg respectively) although it is unclear if the worms guts were emptied prior to analysis. Uptake of VMPs into earthworms potentially exposes a wide range of predators in the environment.

1.5 Effects in the Terrestrial Environment

Veterinary medicines can affect non-target organisms in both the terrestrial and aquatic environment. This section discusses only the effects on terrestrial organisms of parasiticides in particular. Information on aquatic effects can be found in Boxall *et al.* (2004).

Since antibiotics have been specifically designed to be toxic to bacteria and microorganisms in the bodies of animals and humans, it is not surprising these substances affect similar microorganisms in the environment. Sulfonamide antibiotics have been shown to effect microbial growth rate, respiration and nutrient cycling in manured soils (Schauss *et al.*, 2009). For example, in a study performed by Hammesfahr *et al.* (2008), where soils were incubated with manure and sulfadiazine at 10 & 100 mg/kg, microbial growth rate was inhibited and the microbial community structure was altered (a decrease in the ratio of bacteria to fungi was noted) for up to two months after antibiotic addition. A 50% reduction in microbial activity is generally reported at the mid mg/kg concentration range for compounds such as chlortetracycline, oxytetracycline, tylosin and the sulphonamides (e.g. Thiele-Bruhn & Aust, 2004). While these concentrations are relatively high, concentrations of these compounds in slurry are recorded in the high mg/L range and hence there is still potential for soil microbiology to be affected in the field following application of slurry as a fertiliser. However, these effects may be mitigated by the behaviour of these compounds in soil. For example, while chlortetracycline was found to inhibit the growth of 12 soil

bacteria in plate tests, no effect on soil respiration or community structure was observed in soil (Zielezny *et al.*, 2006). In addition to the evidence for the uptake or accumulation of VMs in plants there is also evidence of VMs affecting plant growth and nutrient uptake. For example, chlortetracycline has been shown to cause a reduction in growth of pinto bean plants and to stimulate growth and nitrogen uptake in edible radishes at a concentration of 160 mg/kg (Batchelder, 1982; Batchelder, 1981).

Effects of Parasiticides

The rest of this section will focus on the effects of parasiticides on non-target organisms in the terrestrial environment. Several key parasiticides such as the avermectins and the synthetic pyrethroids are non-specific or broad-spectrum and since they are intrinsically toxic to numerous organisms, they have the potential to be problematic for non-target organisms in the pasture environment.

1.5.1 Dung Fauna

Most of the available data relating to the toxicity of parasiticide residues to dung organisms, presented in Tables 1-5 and 1-6, is in the form of bioassays where manure from treated animals is collected at different times after treatment and the effect on either adult mortality or immature development, often as proportion of introduced eggs or larvae to emerge successfully as adults, is monitored.

Table 1-5 The effect of a range of parasiticides on dung beetles, after Steel *et al.*, (2002)

Drug	Formulation	Species	Adult mortality	Fecundity	Duration of larval mortality (days)	Reference
ABM	SC (cattle)	*Onthophagus binodis*	Yes	Negative	>42	Dadour *et al.*, (2000)
CYP/ CHL	SP	*Copris tripartitus*	Yes (1 day)	Negative (1 day)	No effect on 1st generation, reduced emergence in 2nd generation	Bang *et al.*, (2007)
DOR	Spiked	*Onthophagus gazella*	-	No effect	Yes	Steel & Wardhaugh (2002)
	SC	*Onthophagus binodius*	No	Negative	9-18	Dadour *et al.*, (2000)
EPR	PO	*Onthophagus taurus*	Mortality of newly emerged adults, not mature adults	Negative	7-14	Steel & Wardhaugh (2002)
FLUO	PO (cattle)	*Onthophagus gazella*	No	No effect	No effect	Kryger (2007)
IVM	Oral (sheep)	*Euoniticellus fulvus*	Mortality of newly emerged adults, not mature adults	Negative (1 day)	2-5	Wardhaugh *et al.*, (1993)
	SC (cattle)	*Euoniticellus intermedius*	None	No effect	7-14	Fincher (1992)
		Onthophagus gazella	None	No effect	14-21	Fincher (1992)
		Euoniticellus fulvus	None	No effect	<10	Lumaret *et al.*, (1993)
		Onthophagus gazella	-	No effect	8-16	Sommer *et al.*, (1993)
		Euoniticellus fulvus	-	No effect	7-14	Steel & Wardhaugh (2002)
		Euoniticellus intermedius	-	No effect	14-21	Kruger & Scholtz, (1997)
		Ontis alexis	-	No effect	7-14	Kruger & Scholtz, (1997)

Table 1-5 continued -The effect of a range of parasiticides on dung beetles, after Steel *et al.*, (2002)

Drug	Formulation	Species	Adult mortality	Fecundity	Duration of larval mortality (days)	Reference
IVM	PO (cattle)	*Euoniticellus intermedius*	None	No effect	7-14	Fincher (1996)
		Onthophagus gazella	None	No effect	14-21	Fincher (1996)
		Euoniticellus fulvus	No	No effect	7-14	Steel & Wardhaugh (2002)
		Caccobius jessoensis	-	No effect	7-14	Iwasa *et al.*,(2007)
	SR bolus (sheep)	*Onthophagus taurus*	Mortality of newly emerged adults, not mature adults	Negative	>100	Wardhaugh *et al.*, (2001a)
		Euoniticellus fulvus	No	No effect	>100	Wardhaugh *et al.*, (2001a)
	SR bolus (cattle)	*Onthophagus sagittarius*	Yes	Negative	135	Wardhaugh *et al.*, (2001b)
		Aphodius constans	-	-	143	Errouissi *et al.*, (2001)
MOX	PO (cattle)	Caccobius jessoensis	No	No effect	No effect	Iwasa *et al.*, (2008)

Table 1-6 The effect of a range of parasiticides on dung flies, after Steel et al., (2002)

Drug	Formulation	Species	Duration of larval mortality (days	Reference
ABM	SC (cattle)	*M. vetustissima*	16-32	Wardhaugh & Mahon (1998)
DOR	SC (cattle)	*M. inferior, O. timorensis*	9-15	Wardhaugh *et al.*, (2001b)
	PO (cattle)	*H. irritans, M. domestica, S. calcitrans*	>28	Floate *et al.* (2001)
EPR	SC (cattle)	*M. inferior, O. timorensis*	9-15	Wardhaugh *et al.*, (2001b)
	PO (cattle)	*H. irritans*	>28	Floate *et al.* (2001)
		M. domestica	7-14	
		S. calcitrans	>28	
IVM	Oral (sheep)	*M. vetustissima*	6-8	Wardhaugh *et al.*, (1993)
	Oral (cattle)	*M. vetustissima*	8-16	Wardhaugh & Mahon (1998)
	PO (cattle)	*M. autumnalis*	14	Sommer *et al.*, (1992), Marley *et al.*, (1993)
		M. domestica	7-14	Floate *et al.*, (2001)
		H. irritans	>56	
		S. calcitrans	7-14	
	SC (cattle)	*M. vetustissima*	16-32	Wardhaugh & Mahon (1998)
		M. autumnalis	14	Sommer *et al.*, (1992)
		M. domestica	7	Steel & Wardhaugh (2002)
		M. nevilli	49-56	Kruger & Scholtz, (1995)
	SC (cattle)	*H. irritans*	14	Sommer *et al.*, (1992)
		N. cornicina	10-17	Lumaret *et al.*, (1993)
	SR bolus (sheep)	*M. vetustissima*	100	Wardhaugh *et al.*, (2001a)
	SR bolus (cattle)	*M. inferior, O. timorensis*	115+	Wardhaugh *et al.*, (2001b)
MOX	PO (cattle)	*Haematobia irritans*	7	Iwasa *et al.*, (2008)
		Neomyia cornicina	7	

For the avermectins at least, it appears that excreted residues are generally more toxic to the larvae of dung flies than to the larvae of dung beetles. Ivermectin residues excreted following injection have been observed to remain toxic to fly larvae for up to 32 to 56 days (Sommer *et al.*, 1992; Lumaret *et al.*, 1993; Wardhaugh *et al.*, 1996; Wardhaugh & Mahon, 1998) and beetle larvae for up to 14 to 21 days (Fincher, 1992; Kruger & Scholtz, 1997). However, this does not necessarily mean dung flies are more at risk than beetles. VM residues are in some cases (e.g. abamectin, cypermethrin, doramectin, eprinomectin and ivermectin - see Table 1-5) known be toxic to adult beetles and to suppress beetle oviposition. Many species of beetle are dependent on manure for feeding, whereas flies tend to be dependent on manure for oviposition and larvae development alone. The effect of different application methods described earlier in the introduction is also clearly demonstrated by these studies, where the duration of observed effects falls, with bolus greater than subcutaneous injection which is in turn greater than pour-on.

Given the limited laboratory testing information available, and comparing the same formulation applied to the same animal (injection, cattle), the toxicity of the avermectins to beetle larvae appears to vary with ivermectin toxicity greater than abamectin, which is in turn greater than doramectin and eprinomectin. Comparing the results obtained following pour-on applications suggests moxidectin to be the least toxic to flies. For beetles, the toxicity appears to decrease in the order: abamectin, ivermectin, doramectin. Laboratory toxicity tests conducted by Hempel *et al.,* (2006) comparing the effect of the pour-on formulations suggest that moxidectin appears to be the least toxic of these macrocyclic lactones to beetle larvae. The reported LC_{50} of 4-5 mg/kg (dw) is considerably lower than the peak residue concentrations of 0.6 – 0.8 mg/kg (dw) reported to be excreted from cattle after injection or topical applications (Hempel *et al.*, 2006; Suarez *et al.*, 2009).

Data on the toxicity of organophosphates to dung fauna is more limited. Wardhaugh *et al.,* (2005) indicate that as the organophosphates currently registered for use as parasiticides tend to be extensively metabolised and largely excreted in the urine, therefore these compounds are unlikely to pose a

significant risk to dung fauna. The limited evidence (only two studies) on beetle assays using manure from cattle treated with a topical formulation appears to confirm this, resulting in no effect to very short-lived effects (one day) on adult beetles, and no effect on reproduction (Bang *et al.*, 2007; Kryger *et al.*, 2007).

The insecticidal effect on dung fauna from certain parasiticides has been confirmed by several field studies. For example, in the studies conducted by Floate *et al.*, (1998) artificial pats constructed using the manure from ivermectin treated cattle were allowed to be naturally colonised in the field for a week before being brought in to the laboratory and the resulting insect emergence monitored. A range of organisms were found to be affected by the parasiticide treatment including the coprophagous flies and beetles, parasitic wasps and predacious beetles (Floate, 1998).

1.5.2 Soil Fauna

The assessment of the effects of parasiticides on soil organisms is somewhat limited by the availability of measured concentrations of these compounds in soil. Abamectin concentrations of approximately 1.4 µg/kg (dw) were measured in the pasture soil where sheep were treated with abamectin (Erzen *et al.*, 2005). These concentrations are at least 30 times lower than those recorded to cause a 10% reduction in the reproduction of springtails, *Eisenia foetida* and enchytraeids (Table 1-7). If manure containing high levels of parasiticides is incorporated into the soil then it is possible, but unlikely, that soil organisms will be exposed to residue concentrations affecting survival or reproduction (after dilution with soil) although more data on the transfer of residues to soil is required to accurately assess this risk. Several studies investigating the response of earthworms to parasiticide residues in manure at environmentally-relevant concentrations failed to determine an effect concentration. When tests were performed using manure from cattle or sheep treated with abamectin, doramectin eprinomectin, ivermectin and fenbendazole, no effects on survival or growth rate were detected (Svendsen *et al.*, 2005). Long-term tests performed by Svendsen *et al*., using ivermectin found no effect on the growth rate and survival of either adults or hatchlings. In addition, when the ivermectin-induced effects of small

Table 1-7 The effects of parasiticides on a range of soil organisms

Drug	Test matrix	Species	Adult toxicity concentration (mg/kg)	Effects on reproduction/ juveniles (mg/kg dw)	Reference
ABM	Soil	*Folsomia candida*	LC_{50} 67 (soil) LC_{50} 1.0 (dung)	EC_{50} 13 (soil) EC_{50} 1.4 (dung)	Kolar *et al.*, (2008)
	Soil	*Folsomia candida*	Survival NOEC >2.5 Survival LOEC >2.5 EC_{10} >2.5 EC_{50} >2.5	NOEC 0.25 LOEC 0.50 EC_{10} 0.19 EC_{50} 0.68	Diao *et al.*, (2007)
	soil	*Folsomia fimetaria*	Survival NOEC 0.50 Survival LOEC 1.0 EC_{10} 0.48 EC_{50} 0.81	NOEC <0.25 LOEC 0.25 EC_{10} 0.05 EC_{50} 0.33	Diao *et al.*, (2007)
	Spiked soil, SC manure (sheep)	*Enchytraeus crypticus*	LC_{50} 111 (soil) LC_{50} 1.1 (dung)	EC_{50} 38 (soil) EC_{50} 0.94 (dung)	Kolar *et al.*, (2008)
	Soil	*Enchytraeus crypticus*	Survival NOEC 50 Survival LOEC 150 EC_{10} 78.27	NOEC 10 LOEC 25 EC_{10} 12.7 EC_{50} 23.7	Diao *et al.*, (2007)
	Spiked soil, SC manure (sheep)	*Porcellio scaber* (wood louse)	LC_{50} 69 (soil)	-	Kolar *et al.*, (2008)
	Spiked soil, SC manure (sheep)	*Eisenia andrei*	LC_{50} 18 (soil) LC_{50} >1.4 (dung)	EC_{50} >1.4 (dung)	Kolar *et al.*, (2008)
	Soil	*Eisenia foetida*	Survival NOEC 0.50 Survival LOEC >5.0 EC_{10} >5.0 EC_{50} >5.0	NOEC <0.25 LOEC 0.25 EC_{10} 0.06 EC_{50} 0.39	Diao *et al.*, (2007)
	Soil	*Eisenia foetida*	Biomass NOEC 0.25 Biomass LOEC 0.50 Biomass EC10 0.06 Biomass EC50 0.46	Reproduction LOEC 0.25 Reproduction EC_{10} 0.16 Reproduction EC_{50} 1.03 Hatching NOEC 0.25 Hatching LOEC 0.50 Hatching EC10 0.09 Hatching EC50 0.43	Jensen et al., (2007)

Table 1-7 Continued The effects of parasiticides on a range of soil organisms

Drug	Test matrix	Species	Adult toxicity concentration (mg/kg)	Effects on reproduction/ juveniles (mg/kg dw)	Reference
DOR	Spiked soil, manure (sheep)	*Folsomia candida*	LC_{50} >300 (soil) LC_{50} >2.5 (dung)	EC_{50} 42 (soil) EC_{50} >2.5 (dung)	Kolar *et al.*, (2008)
	Spiked soil, manure (sheep)	*Enchytraeus crypticus*	LC_{50} >300 (soil) LC_{50} >2.5 (dung)	EC_{50} 170 (soil) EC_{50} 2.2 (dung)	Kolar *et al.*, (2008)
	Spiked soil, manure (sheep)	*Porcellio scaber* (wood louse)	LC_{50} >300 (soil)	-	Kolar *et al.*, (2008)
	Spiked soil, manure (sheep)	*Eisenia andrei*	LC_{50} 228 (soil) LC_{50} >2.5 (dung)	EC_{50} >2.5 (dung)	Kolar *et al.*, (2008)
		Earthworms		NOEC 2	Cited in Boxall *et al.*, (2004)
EPR	Manure (cattle, PO)	*Lumbricus terrestris*	No effect on survival, weight gain at 3.34 mg/kg (excreted day 2)	-	Halley *et al.*, (2005)
IVM	Soil	*Folsomia fimetaria*		NOEC 0.3 EC_{10} 1.7 EC_{50} 1.17	Jensen *et al.*, (2003)
	Soil	*Enchytraeus crypticus*		NOEC 3 EC_{10} 14 EC_{50} 36	Jensen *et al.*, (2003)
	Manure (cattle) + soil	*Pristionchus maupasi* (soil nematode)	Population growth: No adverse effect	Hatchling survival: No effect	Gronvold *et al.*, (2004)
	Manure (cattle) + soil	*Lumbricus terrestris*	Growth rate, survival: no adverse effect	Hatchling growth rate, survival: No adverse effect Small decrease in cocoon incubation time & lower growth rate of F1 juveniles No difference in intrinsic growth rate (matrix modelling results)	Svendsen *et al.*, (2005)
	Soil	*Eisenia foetida*	28d LC_{50} 18-100		Halley *et al.*, (1989c)
	Soil	plants	NOEC 0.56		Cited in Boxall *et al.*, (2004)
CYP	Soil	Collembolan	Non toxic		Tomlin (2000)
DEL	Soil	Earthworms	14d LC_{50} 28.6		Tomlin (2000)

Table 1-7 Continued The effects of parasiticides on a range of soil organisms

Drug	Test matrix	Species	Adult toxicity concentration (mg/kg)	Effects on reproduction/ juveniles (mg/kg dw)	Reference
FEN	Manure (cattle)	*Pristionchus maupasi* (soil nematode)	Population growth: No adverse effect		Gronvold *et al.*, (2004)
	Manure (cattle)	*Lumbricus terrestris*	Growth rate: No adverse effect	Hatchling growth rate, survival: no adverse effect No difference in intrinsic growth rate (matrix modelling results)	Svendsen *et al.*, (2005)
	Soil	Earthworms Plants Microbes	NOEC 56 NOEC 36 NOEC 1000		Cited in Boxall *et al.*, (2004)

differences in cocoon development rates and the growth rate of second generation juveniles was assessed using matrix population models, no effect was found on the intrinsic growth rate (Svendsen *et al.*, 2005). These results indicate that avermectin parasiticides pose a low threat to earthworms.

1.5.3 Implications of Reduced Dung Fauna

The removal of key members of the dung fauna community by the use of parasiticides in pastured animals has the potential to slow down the degradation of manure in the field, ultimately leading to a loss of useful pasture and an impact on other organisms in the pasture food chain. The issue of reduced dung degradation in response to parasiticides has been controversial. Several field studies have compared the loss of manure with and without parasiticide treatments, employing different methodologies and producing different results (Barth, 1993). A number of studies found either no effect from parasiticide treatment or reported inconclusive results (e.g. Barth *et al.*, 1993; Wratten *et al.*, 1993; Suarez *et al.*, 2009), while others have reported a significant difference in dung degradation (Floate, 1998; Iglesias *et al.*, 2006; Madsen *et al.*, 1990; Sommer & Bibby, 2002; Wall & Strong, 1987). Support for the impaired dung degradation theory was added by a recent study which confirmed that the physical exclusion of insects for as little as two days after pat deposition can cause a significant reduction in dung degradation rate (Lee & Wall, 2006b).

Another potential impact of parasiticide use is the possible knock-on effect on other organisms in the pasture food chain, i.e. organisms that predate on dung flies and beetles or members of the dung fauna community which benefit from the activity of fly larvae and beetles (Floate & Fox, 1999). Some of the larger dung fauna species, such as aphodius beetles can be an important food source for other pasture animals such as: rooks, choughs, starlings, lapwings, badgers, hedgehogs, shrews, swallows and martins (RSPB, 2006). Further concerns arise from species like the hornet robber fly and greater horseshoe bat, which also predate dung fauna, and which are becoming increasingly rare in the UK.

1.5.4 Field Scale Impacts

The extent to which VMP use causes long-term and/or area-wide damage to local invertebrate populations, either by changing the community composition or by reducing abundance, is more difficult to establish. For example, while the mortality of certain individuals may be unavoidable, it is not clear what level of mortality may be tolerated by the species in question (Barnthouse, 2007). The response of an insect population to VM use is not discernable from laboratory dose-response ecotoxicity tests alone. It will depend on the temporal pattern of exposure - determined by parasiticide excretion rates and livestock treatment regimes in the case of the pasture scenario or degradation rates in storage and field application times in the fertilisation of fields with manure scenario. In addition, the capacity of the population to compensate will be affected by: the reproductive capacity of the population, the possibility of immigration from areas of untreated livestock and the magnitude of other population stressors such as drought and exposure to other chemicals.

There have been a few field studies aimed at addressing the population effects on a wide area and long-term scale. One study performed over two years by Webb *et al.,* (2007), investigated the effects of ivermectin and doramectin treatments in south-west Scotland on the fast-breeding dung fly *Scatophaga stercoraria*. The field study found sub-lethal effects on wing asymmetry were significantly higher in fields of doramectin-treated cattle compared to the control (untreated) fields, but did not detect an effect of avermectin treatment on insect abundance (Webb *et al.*, 2007).

Kruger and Scholtz (1998a; 1998b) performed a two year study in South Africa where two herds were treated with ivermectin and another two left untreated, acting as controls. In the first year, when the area was undergoing a drought, ivermectin was found to affect the dung fauna community structure by reducing species diversity and increasing species dominance. However, in the following year, which experienced higher than average rainfall, no effect of ivermectin on the community was found. The authors suggest that the environmental impact of ivermectin is likely to be determined by several factors, such as climate and the

distance to areas of untreated cattle. Severe climatic conditions, such as the drought experienced in the first year of the study, may act synergistically with parasiticide treatment and hence affect communities more strongly (Kruger & Scholtz, 1998b).

It has been recommended that field investigations into the impact of parasiticide use are conducted on a much larger scale (Wardhaugh, 2005). However, increasing the area under investigation beyond the farm-scale will be difficult to implement due to increased costs and the availability of cooperative neighbouring farms (Wardhaugh, 2005; Floate *et al.*, 2005). Perhaps a more important problem is the timescale of such studies. Subtle changes to reproductive rates due to exposure to toxic compounds may take generations to be measurable (Snell & Serra, 2000). As a result, parasiticide effects on univoltine (one generation a year) beetle populations may not be discernable for several years. Indeed, slower breeding species may be particularly vulnerable to adverse changes in their environment due to their lower reproduction potential (Barnthouse *et al.*, 1990).

One approach is to assess the demographic toxicity, using species-specific data and models to evaluate the impact of a toxicant in terms of population-level endpoints. Ecological modelling is increasingly used as a tool for the risk assessment of xenobiotics on non-target organisms (Meng *et al.*, 2006; Pery *et al.*, 2006; Barnthouse, 2007; Forbes *et al.*, 2008; Snell & Serra, 2000). It may be useful therefore to undertake an assessment of the effect of parasiticide use by population modelling, prior to investing in a large scale, long-term field study.

1.6 Regulatory Assessment of the Environmental Risks of Parasiticides

It is necessary for a Market Authorisation holder to demonstrate the environmental safety of a new VMP before it is authorised to be sold. In order to appropriately assess the risks these products pose to the environment, information on their inputs, fate and effects need to be combined. The VICH guidelines: GL6 Ecotoxicology Phase I (VICH, 2000) and GL38 Ecotoxicology

Phase II (VICH, 2004) and the supporting European Medicines Agency (EMEA) guidance document (CVMP, 2008) are available to guide the risk assessment of new VMPs. The approaches taken in these guidelines are generally very conservative, using large uncertainty factors and simple, easily replicable laboratory tests. However, once an 'unacceptable risk' has been determined for a VMP a better understanding of how these products are behaving in the environment is required. Appropriate methods are required to further refine the risk assessment and to characterize the impact of the VMP on the environment, in particular, methods for extrapolating the effects determined in regulatory laboratory studies to estimate the impacts in the real environment.

For most VMPs, the environmental assessment is performed in two phases. In Phase I, those compounds likely to result in environmental exposure are identified using data on the properties and use of the active substance. For those compounds that do not pass the Phase I assessment, a Phase II assessment is required involving experimental studies. Because of the special concern regarding effects on non-target dung fauna, assessment of parasiticides will go straight to Phase II, where tests are required on the fate of the parasiticide (sorption and persistence), the effects on aquatic and soil organisms and effects on one dung fly and one dung beetle. The Dung Organism Toxicity Testing (DOTTS) group have developed testing strategies for two fly species: *Scatophaga stercoraria* and *Musca autumnalis*. The tests have now been adopted by the OECD as test guidelines 228 and the results of the ring-testing now published (Rombke *et al.*, 2009).

Following the guidelines, the first step in characterising the risk of a parasiticide to dung organisms is to calculate the initial concentration of the parasiticide in the excreted dung ($PEC_{initial\text{-}dung}$) based on the total residue approach. This assumes a worst-case scenario where 100% of the applied dose is excreted in one day entirely as parent compound (no metabolism). The $PEC_{initial\text{-}dung}$ is estimated using Equations 1-1 and 1-2, where A_{VMP} is the mass of active ingredient administered, D is the recommended dose rate for the parasiticide, W is the animal default weight value which is detailed in EMEA guidance document and A_{manure} is the mass of active ingredient in manure (CVMP, 2008).

$$A_{VMP} = D \times W \qquad \text{Equation 1-1}$$

$$PEC_{initial-dung} = \frac{A_{VMP}}{A_{manure}} \qquad \text{Equation 1-2}$$

The risk quotient (RQ) is then calculated using Equation 1-3:

$$RQ = \frac{PEC}{PNEC} \qquad \text{Equation 1-3}$$

Where the PEC (predicted environmental concentration) is calculated in Equation 1-2, and the PNEC is the predicted no-effect concentration which is obtained from the EC_{50} (the concentration causing an adverse effect in 50% of the test organisms) of the dung organism toxicity divided by an assessment factor of 100 (VICH, 2004). If the risk quotient is >1, then the guidance recommends refinement of the PEC, i.e. to calculate the $PEC_{refined\text{-}dung}$ using information on the excretion rate and metabolism in the target animal and biodegradation information from manure/soil systems to estimate predict a more realistic concentration to which to compare the effects data. This will be explored in Section 1.8. If at this stage of the risk assessment, the RQ is still >1 then the guidance for VMP for pastured animals does not recommend additional studies (as it does for other, non-parasiticide VMP for non pastured animals), but suggests that regulatory guidance should be sought. However, as this is an emerging area, the recommended further work to refine the risk assessment is still unclear and under investigation.

The equations above are likely to provide a highly conservative assessment of the risks of a parasiticide to dung organisms. In the real environment, it is likely that a catchment will contain both non-contaminated and contaminated dung; that the residues of the parasiticide in the dung will vary (over time from treatment and the possibility of degradation of residues in the environment); that dung

organisms may or may not come into contact with contaminated dung (depending on the life history of the organism and the timing of treatment and husbandry of the animals); and that even when organisms are affected they may have the potential to recover. It is therefore necessary to develop improved approaches for more accurately assessing the risks from parasiticides to dung organisms, so that the health benefits from the use of veterinary medicines can be realised whilst ensuring that the impact of parasiticides on the environment is acceptable.

1.7 Overall Aim of the Project

This PhD was part of a European project called ERAPharm, the aim of which was to explore higher tier approaches for the risk assessment of human and veterinary medicines. As described in the previous section, there is currently a lack of guidance on how to refine the risk assessments of parasiticides, the group of veterinary medicines used to treat pastured livestock for internal and/or external parasites. Dung fauna in particular, are potentially at risk from these treatments, but other than recommending the toxicity testing of the larvae of a dung beetle and fly, little guidance is available for the next steps in the risk assessment if the initial risk assessment indicates non-target pasture fauna are at risk.

This PhD was aimed at the exploration of higher tier methods for refinement of the risk assessment of parasiticides. The central hypothesis of the work was that the effects of a parasiticide on populations of dung organisms can be estimated or predicted based on 1) the results of standardised toxicity tests and 2) fate studies performed under field conditions such as would be either available or undertaken by industry.

The parasiticide ivermectin was selected as the case-study compound for this series of investigations for a number of reasons. Firstly, it is one of the most commonly used parasiticides worldwide and the most commonly used parasiticide in the UK, (Boxall *et al.*, 2007). Secondly, there is potential cause for concern for this parasiticide as it has been proven to be excreted at levels

toxic to dung degrading and other pasture fauna (see Tables 1-5 and 1-6). However, its impact at the field scale is not well understood. In particular, its fate under pasture conditions, a key consideration in the refinement of risk assessments, is unclear. Standardised laboratory degradation studies can give an indication of fate in the environment but the range of data available from field studies between complete degradation of residues in manure in 6 days, to no degradation apparent after 180 days (Suarez *et al.*, 2003; Lumaret *et al.*, 1993), suggest that fate under field conditions is not so easily predicted.

The overall aim of this project was to explore and develop improved approaches for estimating the risks of veterinary parasiticides in the terrestrial environment. This was undertaken using the following specific objectives:

1. To develop and validate methods for measuring concentrations of a case study parasiticide in soil, surface waters and manure.
2. To explore the fate of that case study parasiticide under field conditions using those methods.
3. To develop a methodology for assessing the risks of parasiticides to terrestrial organisms based on drug use, laboratory toxicity data, fate data and the ecological characteristics of the species of interest
4. To utilise the methodology using the case study parasiticide as an example.

The main hypotheses of the PhD were as follows:

- Suitable methods for measuring the concentrations of a case-study compound can be developed.
- Those methods can be used to develop a better understanding of the inputs of the case study compound to the various compartments of the terrestrial environment.
- A simple population model based on the Leslie matrix model can be used to produce an illustrative assessment of the potential risks posed to dung fauna.

Further details of the specific hypotheses for each chapter will be introduced later.

1.8 Introduction to the Case Study Compound: Ivermectin

The parasiticide ivermectin was selected as the case-study compound for this series of investigations exploring the fate of parasiticides under pasture conditions. Although ivermectin has been proven to be excreted at levels toxic to dung-degrading and other pasture fauna, its fate under pasture conditions and the impact of its use on the field scale is not well understood. It would therefore be useful to research: 1) fate ivermectin under pasture conditions and 2) field-scale impacts of ivermectin usage.

Concerns regarding the impact of ivermectin residues from treated livestock on non-target pasture fauna and the potential environmental consequences were first raised in 1987 (Wall & Strong, 1987). Since then the direct and indirect effects of ivermectin on dung and soil fauna have been extensively studied. The existence of this large body of toxicity data that we may build upon makes ivermectin an ideal compound for this study into the risk assessment of parasiticides in the pasture environment.

Ivermectin is one of the avermectins, a group of macrocyclic lactones derived from the fermentation products of the soil organism *Streptomyces avermitilis* (Campbell *et al.*, 1983). Ivermectin is a synthetic derivative of the naturally occurring avermectin B_1 and was the first macrocyclic lactone to be developed for use in veterinary medicine (Shoop & Soll, 2002). The avermectin group have a unique pharmacophore responsible for their anti-parasitic control (Shoop & Soll, 2002), illustrated in Figure 1-3. This pharmacophore consists of a 16-membered macrocyclic backbone which is responsible for their mode of action. Ivermectin is made up of 2 components: at least 80% of component B_{1a} (5-O-demethyl-22,23- dihydroavermectin A_{1a}) and up to 20% ivermectin component B_{1b} (Bloom & Matheson, 1993).

Ivermectin and the avermectins group in general are broad spectrum parasiticides, active at very low doses against a wide range of nematode and arthropod parasites of animals (Campbell *et al.*, 1983). Ivermectin is used worldwide as anti-parasitic medicine for livestock. In the UK, ivermectin formulations are authorised for use in cattle, sheep, pigs, horses and household pets (NOAH, 2008). It works as both an endo- and an ectocide, controlling both internal and external parasites. Ivermectin's strong efficacy, apparent safety, and ease of use make it a popular choice for livestock farmers. A recent survey found ivermectin to be the most commonly used parasiticide for cattle (Boxall *et al.*, 2007), with approximately 50% of the cattle farms surveyed using ivermectin. In the UK ivermectin is licensed for use in UK pasture animals in different formulations (see Table 1-8). This study will focus on a scenario of pastured cattle.

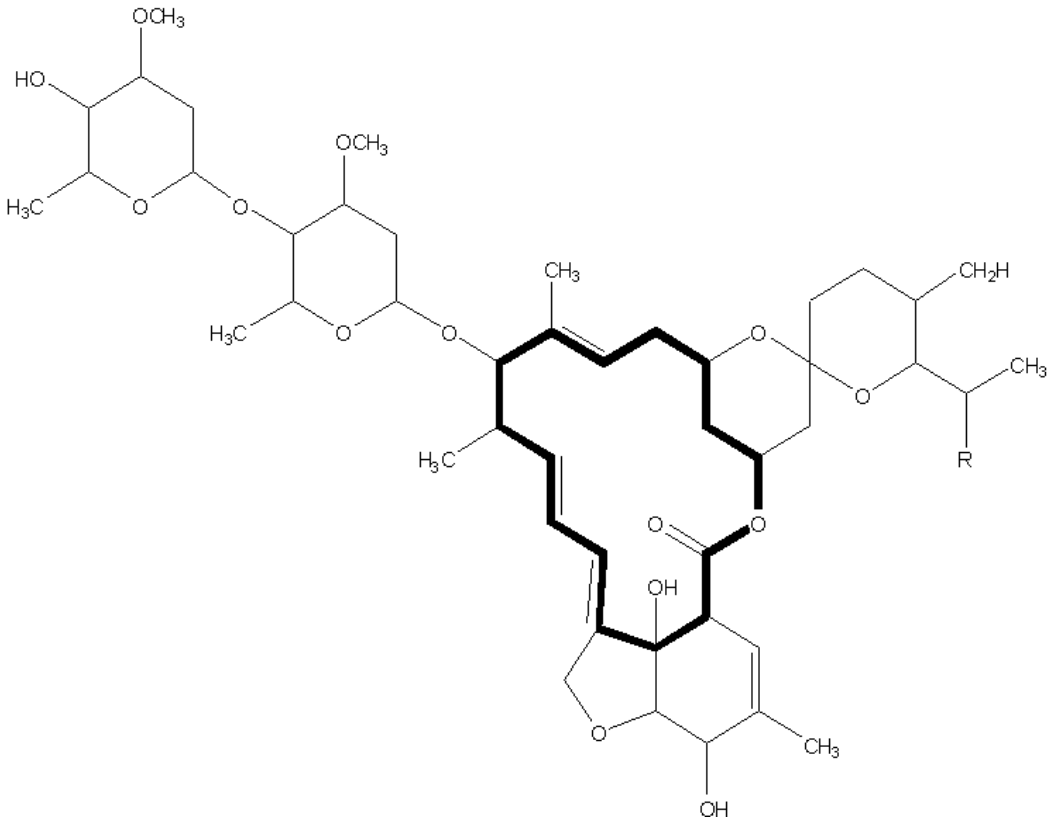


Figure 1-3 Structure of ivermectin with the pharmacophore responsible for anti-parasitic effects in bold. R = C_2H_5 in H_2B1_a and R = CH_3 in H_2B1_b (Shoop & Soll, 2002)

Table 1-8 Ivermectin formulations available in the UK for pasture livestock (NOAH, 2008)

Formulation	Cattle	Sheep	Horses	Pigs
Subcutaneous injection (non-aqueous 1% w/v, 10mg/ml)	X	X		X
Pour-on (non-aqueous 0.5% w.v)	X			
Oral drench (aqueous 18.7 mg/g, 0.08% w/v, 1.55% w/w)		X	X	
Pre-mix meal mixture (0.6% w/w)				X

The avermectins have large molecular weights and are very hydrophobic compounds (Table 1-9). They exhibit very low water solubility and are largely involatile. The high K_{oc} (and low water solubility) of the avermectins indicate a tendency to sorb strongly to organic matter. This suggests avermectin residues will not be easily leached from the organic-rich manure into the soil.

Table 1-9 Avermectin characteristics K_{oc} = soil/water partition coefficient, K_{om}= organic matter partitioning coefficient, K_d= sorption coefficient, K_{ow}= octanol/water partitioning coefficient

	Abamectin	Ivermectin	Doramectin
Composition		>80% B_{1a}: $C_{48}H_{74}O_{14}$ <20% B_{1b}: $C_{47}H_{72}O_{14}$	
Molecular mass (g/mol)		B_{1a}: 875.1 B_{1b}: 861.1	
Melting point		150 ºC	
Molecular mass (g/mol)		874.7	
Water solubility	7.8 ppb	4 ppm[1]	25 ppb
K_d	Avm B1a: 80 (silt loam soil) Avm B1a: 147 (clay loam soil)	333 (clay loam soil, exp) 227 (silty clay loam soil, exp)	15,600 (manure) 70.8 – 562 (soils)
K_{oc}	4,000[2] 5300-15700	12,600 and 15,700 L/kg	34,100 (manure) 7,520 – 86,900 (soils)
K_{om}		4500 to 5500 L/kg	
Log K_{ow}		3.21[1]	4.77
Vapour pressure (Pa)		< 1.5 x10^{-9}mm Hg	

Bloom & Matheson (1993), Wislocki *et al.,* (1989), Halley *et al.*, (1989a), Hempel *et al.,* (2006), Pfizer (1996), Merial (1990)

Standard Phase II Assessment for Effects of Ivermectin on Dung Organisms

Using the available data on the use and ecotoxicity of ivermectin, it is possible to perform a standard Phase 2 assessment of the concentration of ivermectin in dung and the risk to dung communities using Equations 1-1 to 1-3 described in Section 1.6 above.

Using the recommended dose rate for an ivermectin subcutaneous injection of 200 µg per kg cattle body weight (NOAH, 2008), the animal default values in EMEA for body weight of a beef bullock (330 kg) and a manure excretion rate of 13 kg manure per day (CVMP, 2008), the concentration is calculated according to Equations 1-1 and 1-2 above to yield the results shown in equations 1-4 and 1-5:

$$Ivermectin\ per\ animal = 200\ \mu g \times 330\ kg = 66\ g \qquad \text{Equation 1-4}$$

$$PEC_{initial-dung} = \frac{66\ g\ ivermectin}{13\ kg\ manure} = 5\ mg/kg\ ww \qquad \text{Equation 1-5}$$

The EC_{50} for ivermectin on dung flies is 18 µg/kg ww (Rombke *et al.*, 2009), dividing this by the recommended assessment factor of 100 (VICH, 2004) gives a PNEC of 0.18 µg/kg ww. Assessment (or safety) factors are used in risk assessment for the calculation of PNECs to reflect the uncertainties in predicting ecosystem effects from laboratory data. For a particular environmental compartment (e.g. soil), if toxicity information is available from multiple, relevant trophic levels and taxonomic groups, and more sensitive, long-term endpoints are available (such as effects on reproduction) the confidence in the relevance of the data set increases and a reduction in the assessment factor may be justified.

Calculation of the risk quotient according to Equation 1-3 results in a RQ of around 28,000 as shown in Equation 1-6:

$$RQ = \frac{PEC}{PNEC} = \frac{5000\ \mu g/kg\ \ fw}{0.18\ \mu g/kg\ \ fw} = 27{,}778 \qquad \text{Equation 1-6}$$

This very high risk quotient indicates that, using this initial highly-conservative risk assessment method, ivermectin is likely to pose an unacceptable risk to dung-dwelling organisms. As mentioned in Section 1.6 a risk quotient of greater than 1 requires further investigation to demonstrate safety. This safety could be achieved by demonstrating that a more accurate risk assessment shows that the compound will not have an impact on non-target organisms and/or introducing risk management strategies to reduce the risk sufficiently.

In the following chapters a series of investigations are described and a model is developed to better understand the risks of ivermectin. In Chapter 2 analytical methods are developed to determine ivermectin levels in various matrices and then in Chapter 3 these methods are used to investigate the excretion profile of ivermectin from treated cattle in the pasture environment along with a mesocosm study into the fate of the case compound in the aquatic environment. Chapter 4 uses this more accurate environmental input data to build an illustrative computer model of how realistic ivermectin exposure can affect dung-degrading organisms. Finally, Chapter 5 discusses the broad implications of this work and provides recommendations for future research.

While the work has focused on ivermectin, the results are also relevant to other veterinary parasiticides.

2 Analytical Methods

In order to investigate the impact of parasiticide use (in this case ivermectin) on non-target pasture organisms, a clear understanding of the amounts reaching the field over time is required coupled with information on its fate over time. In Chapter 3, two field studies are presented that investigate the fate of ivermectin in the environment to generate these data. A UK field study was designed and performed to measure the excretion rate from treated animals, degradation of residues in the field and occurrence in soil following treatment. Secondly, a mesocosm study in Canada was supported by measuring the dissipation of residues in water and sediment (Sanderson *et al*., 2007).

In order to perform these studies it was first necessary to develop and validate suitable methods to quantify residues in various environmental matrices (such as dung and soil). These methods generally involve extraction of the analyte from the matrix followed by a clean-up or purification step before chromatographic separation and detection. Initially, the methods used by previous authors for avermectin analysis were reviewed in order to assess which general approach would be suitable. Once a general approach was selected, the methods were optimised for the specific matrices (e.g. manure, soil) involved in this project and fully validated.

This chapter will discuss previously published methods for the determination of avermectin residues in environmental samples; briefly describe the optimisation considerations and the methodology used by this study; and report the validation results relating to the extraction methodologies proposed.

2.1 Introduction

This section will briefly review the separation, detection, extraction and clean-up methods used in the literature for the determination of avermectin residues in environmental matrices.

Ivermectin is likely to be encountered at concentrations in the mg/kg (dw) range in manure (Table 2-1). Therefore, methods achieving limits of detection significantly lower than this will be suitable for manure analysis. Although there have been no reports of ivermectin in the soil compartment under field conditions following the use of ivermectin as a parasiticide, abamectin has been detected in the low µg/kg range in the pasture of abamectin treated sheep (Erzen *et al.*, 2005). The only report of ivermectin levels detected in the sediment of fresh-water systems following livestock treatment were also in the low µg/kg range (Boxall *et al.*, 2006a). The methods of detection developed in the following sections will therefore need to be sufficiently sensitive to quantify residues at these concentrations.

Table 2-1 Examples of maximum measured concentrations in different environmental matrices

Matrix	Concentration detected (mg/kg dw)	Reference
Manure	Peaks of 18.5 after pour-on, 1.2 after subcutaneous injection (ivermectin)	Herd *et al.*, (1996)
Soil	Peak of 0.0014 in sheep grazed pasture (abamectin)	Erzen *et al.*, (2005)
Sediment	0.00491, cattle pasture (ivermectin)	Boxall *et al.*, (2006a)

2.1.1 Separation and Detection Methods

A number of separation and detection methods have been employed in the analysis of avermectin residues, both for screening and analyte confirmation and for quantitation of residues.

Thin layer chromatography has been used for the screening of dung samples for the presence of ivermectin residues (Floate *et al.*, 1997). While this method is rapid, its sensitivity and selectivity is poor compared to other methods with reported limits of detection of 40 µg/kg wet dung (Floate *et al.*, 1997).

Liquid chromatography tandem mass spectrometry (LC-MS) has been the preferred technique for confirmation of residue identity due to its inherent specificity and sensitivity (Ali *et al.*, 2000a). In addition, LC-MS has been increasingly used in the quantification of avermectin residues (see Table 2-2). A

number of different ionisation sources have been applied to macrocyclic lactone (ML) residues, including particle beam, thermospray, electrospray (ES), atmospheric pressure chemical ionisation (APCI) and atmospheric pressure photo-ionisation (APPI) (Danaher *et al.*, 2006).

While particle beam and thermospray have been used for the confirmation of avermectin identity (Heller & Schenck, 1993; Afzal *et al.*, 1994), electrospray (Sheridan & Desjardins, 2006; Durden, 2007; Hou *et al.*, 2006; Yoshii *et al.*, 2004; Turnipseed *et al.*, 2005; Pozo *et al.*, 2003; Croubels *et al.*, 2002), APCI and APPI (Brewer *et al.*, 2004; Howells & Sauer, 2001; Turnipseed *et al.*, 2005; Wu *et al.*, 2001) have been used for quantitation. However, some authors have reported difficulties using these methods. The matrix may interfere with the ionisation efficiency resulting in either signal suppression or enhancement (e.g. Brewer *et al.*, 2004). When the positive mode is used, the avermectins tend to form sodium adducts which can be unpredictable in formation and can lead to variable results, and may be difficult to fragment quantitatively (e.g. Stout *et al.*, 2000). The mobile phase may be modified to include a source of ammonia in order to force the formation of the more reliable NH_4^+ adduct (Hernando *et al.*, 2007). Finally, several different mass analysers have been used to fragment the avermectins including the quadrupole, triple quadrupole, time-of-flight (TOF), and ion trap (Danaher *et al.*, 2006). These methods can result in suitably low limits of detection (Table 2-2).

High performance liquid chromatography (HPLC) has been extensively used in the determination of avermectin residues in various matrices, with both UV and fluorescence detection. Earliest methods for determining avermectin residues used UV detection, a simple, rapid and robust method that can be readily applied to avermectins due to their strong UV chromophore (Danaher *et al.*, 2006). While HPLC analysis with UV detection is an adequate method for samples with very high avermectin levels such as medicated feed (Doherty *et al.*, 1998) it often offers limited sensitivity and selectivity and is therefore highly dependent on extensive clean-up procedures (e.g. Lumaret *et al.*, 1993; Bernal *et al.*, 1994). Reported limits of detection range from 10-20 µg/kg (probably wet weight) of manure (see Table 2-3).

Table 2-2 LC-MS/MS ionisation and detection methods for avermectin analysis, concentrations were not specified as on a dry or wet weight basis

Ionisation Method	Detection method	LOD/Q (µg/kg)	Matrix	Matrix and Reference
ESI in negative and positive mode (detection of $[M^+Na]^+$ adduct)	Unknown	LOQ: 0.57-0.84 (- mode) 0.18-0.93 (+ mode)	Milk	Durden (2007)
	Triple-quadrupole	LOD: 2.5	Liver	Hou *et al.*, (2006)
APCI in negative mode	Triple-quadrupole	LOQ 0.4	Sediment, water	Loffler and Ternes (2003)
Turbo ion spray in positive mode (detection of NH_4^+ adduct)	Hybrid quadrupole/linear ion trap (QqQ_{LIT})	ILQ: 0.15-5	vegetable	Hernando *et al.*, (2007)

Table 2-3 HPLC methods using UV detection, concentrations were not specified as on a dry or wet weight but likely to be on a wet weight basis

Analyte	Sample Matrix	LOQ/ LOD	HPLC Column	Mobile Phase	Reference
IVM	Cattle dung	LOD: 10 µg/kg LOQ: 200 µg/kg	*C18, 300 x 3.9mm*	ACN/ methanol /water (47/33/20)	Bernal *et al.*, (1994)
IVM	Cattle dung	20 µg/kg	*C18*	ACN/ methanol /water	Lumaret *et al.*, (1993)
ABM	Lettuce and cucumber	LOD: 40 µg/kg	*C8, 5 µm*		Vuik *et al.*, (1991)

More recently, HPLC with fluorescence detection (FD) has been used for the analysis of avermectin residues (see Table 2-4). Fluorescence detection can be extremely sensitive and selective, typically three orders of magnitude more sensitive than UV detection (Snyder *et al.*, 1997). Since ivermectin does not naturally fluoresce, derivatisation of the molecule is required. In the derivatisation reaction, the dihydroxycyclohexene ring of the avermectins is dehydrated in the presence of catalysts to produce an intensely fluorescent derivative (Sams, 1993). Derivatisation is usually performed using trifluoroacetic anhydride (TFAA) in the presence of n-methylimidazole as the catalyst, illustrated in Figure 2-1 (e.g. DeMontigny *et al.*, 1990). The reaction is almost instantaneous at ambient temperature for most avermectins, with the exception of eprimomectin which requires temperatures of approximately 65 ºC (Ali *et al.*, 2000b).

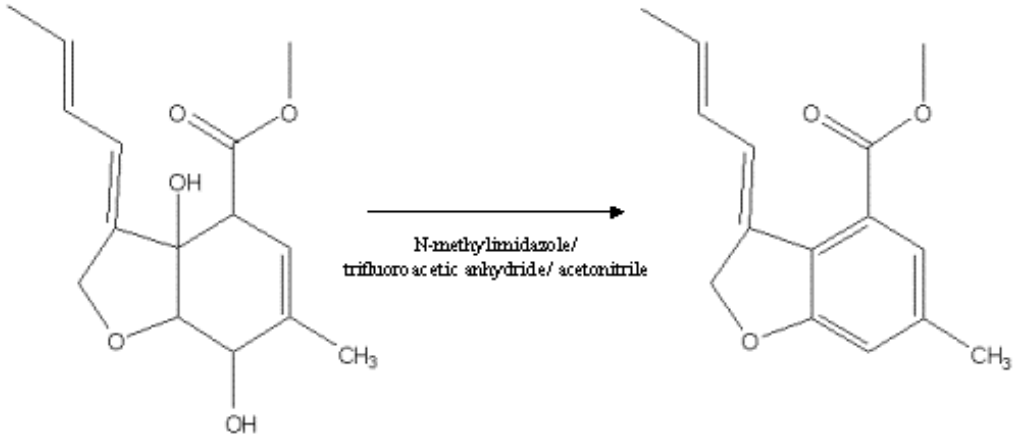


Figure 2-1 Formation of the fluorescent ivermectin derivative (Payne *et al.*, 1995; DeMontigny *et al.*, 1990)

The disadvantages of this method include the necessity of removing all water prior to derivatisation (which would otherwise interfere with the dehydration of the

avermectin molecule) and the low stability of the derivative: 24h at 4°C and 10h at room temperature (DeMontigny *et al.*, 1990).

The fluorescent avermectin derivatives are typically separated by reverse-phase chromatography using either C8 or C18 columns (in many cases after using a pre-column for further clean-up) and using a mobile phase of acetonitrile (ACN) and/or methanol and water, either isocratically or using a gradient. In some cases acetic acid has also been added to the mobile phase (e.g. Lifschitz *et al.*, 2000; Perez *et al.*, 2001; Lumaret *et al.*, 2005) to further aid separation. The limits of detection (LOD) and quantitation (LOQ) achieved for manure analysis range from approximately 0.5 µg/kg to 1 mg/kg and from 0.5 to 2.5 µg/kg respectively (see Table 2-4). Limits of detection achieved for soil analysis are typically lower with LODs ranging from 0.1 to 1.5 µg/kg reported (Table 2-4). This is may be due to fewer interfering peaks co-extracted with the analyte in the soil matrix.

2.1.2 Extraction

There are numerous methods for the extraction and analysis of avermectin residues from biological samples such as liver, plasma, milk and muscle (e.g. Ali *et al.*, 2000b; Roudaut, 1998; DeMontigny *et al.*, 1990; Danaher *et al.*, 2001). These matrices have their own analytical considerations (for instance methods may involve a step for removing fats and proteins) and are therefore not discussed here. Table 2-5 summarises published methods of avermectin extraction and sample clean-up for matrices relevant to this study, such as faeces, soil, sediment and water.

Most avermectin extraction methods employ solid-liquid extraction (SLE) with solvents, a simple and straightforward method of extraction which after subsequent clean-up steps can be sufficiently sensitive and selective for environmental analysis of avermectins. Published methods using SLE have achieved recoveries and limits of detection of approximately 80 – 100% and 0.5 – 1 µg/kg respectively (see Table 2-5).

Table 2-4 HPLC methods using fluorescence detection

Analyte(s)	Sample Matrix	LOQ/ LOD µg/kg	HPLC Column	Mobile Phase	Reference
ABM	Dates	5	C18 0.15 x 4.6mm	ACN/Water (96/4 v/v) 1.5ml/min flow rate, 50ul injection volume	Kamel *et al.,* (2007)
ABM, DOR, EPR, IVM	Cattle liver and muscle	LOD 0.5-1.0 LOQ 1-2	C18 4.6 x 250 mm id, 5µm particle size	ACN/water/THF (88/4/8 v/v/v)	Hou *et al*., (2007)
EPR	Cattle dung	LOD: 1000 wet weight	250 x 4.6 mm, packed with suplex pKb 100 (5µm) material,	(0.4%) acetic acid-Pic B7-acetonitrile (42:0.4:57 v/v/v) 1ml/min flow rate	Lumaret *et al.,* (2005)
ABM	Earthworms	LOD 0.5	C18 5um, 250x4.6mm	methanol/water 97/3 v/v, 100ul injection volume	Sun *et al*., (2005)
ABA, DOR	Sheep dung	LOD: 1.0 ww LOQ: 2.5	C18 150x4.6mm id, 3µm particle size	methanol/ACN/Water (47.5/47.5/6)	Kolar *et al*., (2004)
ABM, DOR	Soil	ABM then DOR LOD dry soil:0.7, 1.5 LOQ dry soil: 1.0, 2.5	LC-8-DB 250 x 4.6mm id, 5µm particle size	methanol/acetonitrile/water (47.5/47.5/6), flow rate 1.1ml/min,	Erzen and Flajs (2003)
IVM, MOX	Horse dung	LOQ: 0.5	C18 3µm column 150 x 4.6mm	0.2% acetic acid/methanol/ACN (4/32/64 v/v/v)	Perez *et al.,* (2001)
IVM, DOR	Dung, tissue and fluids	LOQ: 0.1-100	C18 3µm column 150 x 4.6mm	0.2% acetic acid/methanol/ACN (4:32:64 v/v/v) 1.5ml/min flow rate	Lifschitz *et al*., (2000)
ABM	Fruit & vegetables	Lowest level validated: 10	C18, 12.5cmx4mm 5µm particle size	ACN/water (94/6 v/v) 1.5ml/min flow rate, 50µl injection volume	Diserens and Henzelin (1999)
IVM	Reindeer dung	Lowest tested: 5 ww			Asbakk *et al.*,(1999)
ABM	Foliage, litter, soil, sediment	LOD LOQ µg/kg Foliage 0.20 - 0.60 Litter 0.20 - 0.60 Soil 0.10 - 0.30 Sediment 0.10 - 0.30	C18, 250 x 4mm id, 5µm particle size	methanol/water, 100ul injection volume	Sundaram and Curry (1997)
ABM	Hops	LOD: 2 LOQ: 5	C18 150 x 4.6mm, 3um particle size		Cobin and Johnson (1996)
IVM	Cattle dung		C18, 150 x 4.6mm id, 3µm particle size	5%water in methanol 1.2ml/min flow rate, 50µl injection volume	Payne *et al.,* (1995)
IVM	Cattle dung	LOD: 10 LOQ: 200	C18 300 x 3.9mm id,	mobile phase ACN/methanol/water 47/33/20	Bernal *et al.,* (1994)
IVM	Cattle dung	LOD: 50 dw (0.05ppm) corresponding to 0.37 ng/ml in the injected sample	C18 150 x 3.9 mm, 5µm particle size	acetonitrile/methanol/water (56/37/7 v/v/v) 1.5ml/min flow rate, 20µl injection volume	Sommer *et al.,* (1992)
EMA, IVM, ABM, MILB	Crops	0.0001-0.0003	150x4.6mm id Wakosil-II 3C188HG column 3µm particle size	Solvent A: acn, sol B: water. Initially 80-20%, to 90-10% over 5 minutes, 93-7 over 20 min, 100 over 2 min (total 27 minutes)	

Other extraction methods used in the analysis of the avermectins include matrix solid phase dispersion (MSPD) and accelerated solvent extraction (ASE) (see Table 2-5). Matrix solid phase dispersion combines analyte extraction and sample clean-up into one step, reducing the volume of solvent used and time required for sample preparation and has been used to achieve 94 – 99% recovery and an LOQ of 2.5µg/kg (Valenzuela *et al.*, 2001; Barker, 2007; Brewer *et al.*, 2004). Accelerated solvent extraction has the advantage of being an automated method once the method has been developed and usually requires smaller solvent volumes. Recoveries of up to 91% have been achieved using ASE. However, method development of such a sensitive system may be time consuming.

Commonly used solvents for extraction (e.g. for SLE, ASE or MSPD) include acetonitrile (Diserens & Henzelin, 1999; Lifschitz *et al.*, 2000; Loffler & Ternes, 2003; Perez *et al.*, 2001; Lifschitz *et al.*, 2000; Kolar *et al.*, 2004; Lumaret *et al.*, 2005; Sun *et al.*, 2005; Diserens & Henzelin, 1999; Kamel *et al.*, 2007), acetone (Loffler & Ternes, 2003; Erzen & Flajs, 2003; Yoshii *et al.*, 2001; Yoshii *et al.*, 2004), ethanol, and methanol (Floate *et al.*, 1997; Brewer *et al.*, 2004), either alone or in a mixture with water, ethyl acetate (Sundaram & Curry, 1997; Loffler & Ternes, 2003) or dichloromethane. Table 2-5 reports the recoveries and limits of detection achieved using these methods. However, it is difficult to attribute differences to the extraction solvent alone because recovery and detection limits will be influenced by extraction methods (e.g. SLE, ASE, MSPD) and subsequent sample clean-up and detection.

2.1.3 Sample Clean-up

Following extraction it is often necessary to undertake clean-up steps to remove matrix interferences. This may be liquid-liquid partitioning between solvents, filtering, solid-phase extraction (SPE) or in many cases a combination of these (see Table 2-5).

In the SPE of avermectins, three types of phases are used: ion exchange, normal phase (NP), and most commonly, reverse phase (RP). Ivermectin is a neutral, high

molecular weight, hydrophobic compound and therefore amenable to reverse phase (RP) chromatography, once extracted into a polar organic solvent such as acetonitrile (ACN) and diluted with water. For the analysis of avermectins the most commonly used RP sorbent types is octadecyl (C18) bonded silica (e.g. Sommer *et al.*, 1992; Payne *et al.*, 1995; Asbakk *et al.*, 1999; Perez *et al.*, 2001; Lifschitz *et al.*, 2000; Yoshii *et al.*, 2001; Lumaret *et al.*, 2005; Sun *et al.*, 2005; Loffler *et al.*, 2005; Diserens & Henzelin, 1999; Kamel *et al.*, 2007; Yoshii *et al.*, 2004). Other sorbent types have also been used for the clean-up of avermectins such as silica (Brewer *et al.*, 2004; Kamel *et al.*, 2007), alumina (Sun *et al.*, 2005; Hernando *et al.*, 2007), aminopropyl bonded silica (Yoshii *et al.*, 2001), florisil (Sundaram & Curry, 1997) and primary and secondary amine (PSA).

2.1.4 Literature Methods Conclusion

The choice of extraction method depends on a number of factors including: analyte characteristics, sample matrix, residue levels likely to be encountered and measurement method to be used. Choice of measurement method will also depend on required sensitivity, cost and availability of instrumentation.

The analytical method selected for this study was based upon the results of the literature review and the availability of instrumentation. HPLC–FD was available and the literature review showed it could be highly effective in the determination of residues. Solid-liquid extraction and C18 SPE were selected since it was relatively straightforward to use and the review showed it could be effective. These methods were then optimised for the different matrices to be analysed in this project.

Table 2-5 Avermectin extraction and clean-up methods in the literature for environmental matrices

Analyte/s	Sample Matrix	Extraction	Clean-Up	Detection Method	LOD/ LOQ	Recovery (%)	Reference
ABM	Dates	SLE using 80% ACN	C18 SPE followed by silica SPE	HPLC-FD (quantitation)	LOD: 5 µg/kg	92 - 101	Kamel *et al.*, (2007)
ABM, IVM, EMA, DOR	Salmon and pepper	SLE using ACN (0.1% acetic acid)	Alumina (Al-N) SPE	LC-MS/MS (quantitation and confirmation)	ILQ 0.15-5 ppb	80 - 95	Hernando *et al.*, (2007)
ABM	Earthworm	SLE using ACN	Alumina SPE followed by C18 SPE. Samples filtered after derivatisation	HPLC-FD (quantitation)	LOD: 0.5 µg/kg	83 -105	Sun *et al.*, (2005)
EPR	Cattle dung	SLE using ACN/water (2:1)	C18 SPE	HPLC-FD (quantitation)	LOD: 1mg/g (ww)	63.4- 81.4	Lumaret *et al.*, (2005)
ABM DOR	Sheep dung	SLE using ACN	C8 SPE followed by LLE with n-hexane	HPLC-FD (quantitation)	LOD: 1.0 µg/kg (ww) LOQ: 2.5 µg/kg (ww)	66 - 81	Kolar *et al.*, (2004)
ABM, EMA, IVM	Fruit and Vegetables	SLE using ACN	SPE using PSA (primary/secondary amine)	LC-MS (quantitation and confirmation)	LOD: <4 µg/kg LOQ: <7 µg/kg	82 - 109	Zywitz *et al.*, (2004)
ABM	Soil	ASE using dichloromethane	Silica gel SPE	LC/MS/MS (quantitation and confirmation)	Not given	68% using LC-MS/MS and	Brewer *et al.*, (2004)
MIL IVM ABM EMA and EMA metabolites	Tomato, Japanese radish & Tea	SLE using acetone	C18 SPE	LC-MS (confirmation of MIL, IVM & ABM, quantitation of EMA and EMA metabolites)	0.1- 0.5 ng/ml (10 µl injection)	90 - 120 for EMA	Yoshii *et al.*, (2004)
IVM	Sediment	SLE using acetone/ACN (20:1), followed by ethyl acetate	Filtered, followed by C18 SPE	LC tandem MS (quantitation and confirmation)	LOQ: 0.4 µg/kg	31± 6 and 41 ± 16% for 2 spike levels (20 and 3 ng/g), 102 ±20, 119±14 after compensation with ABM	Loffler and Ternes (2003)
ABM, DOR	Soil from grazed pasture	SLE with acetone/water (1/1)	LLE using isooctane followed by alumina SPE	HPLC-FD (quantitation)	LOQ: 1.0 and 2.5 µg/kg (dw) for ABM and DOR respectively	70.6 - 96.3	Erzen and Flajs (2003)
ABM	Citrus fruit	Matrix solid-phase dispersion (MSPD) using C18 bonded silica		LC-ES-MS (quantitation and confirmation)	LOQ: 2.5 µg/kg	0.001-10mg/kg range 94-99%, average rec 96%	Valenzuel *et al.*, (2001)
IVM, MOX	Horse dung	SLE using ACN/water (2:1)	C18 SPE	HPLC-FD (quantitation)	LOQ: 0.5 µg/kg	72.4±9.3% for mox, 69.15±9.01% for IVM	Perez *et al.*, (2001)
EMA & Mets, IVM, ABM, MIL	Crops	SLE using acetone	C18 and NH_2 SPE	HPLC-FD (quantitation)	0.1- 0.3 ppt (trillion)	80-110	Yoshii *et al.*, (2001)

Table 2-5 continued - Avermectin extraction and clean-up methods in the literature for environmental matrices

Analyte/s	Sample Matrix	Extraction	Clean-Up	Detection Method	LOD/ LOQ	Recovery (%)	Reference
IVM DOR	Tissue, fluid and dung samples	SLE using ACN	C18 SPE	HPLC-FD (quantitation)	LOQ: 0.1 µg/kg	>72% over 0.1-100 ng/g range	Lifschitz *et al.*, (2000)
ABM	Fruit and vegetables	SLE using ACN	C18 SPE	HPLC-FD (quantitation)	LLV: 10 µg/kg	88 - 106	Diserens and Henzelin (1999)
IVM	Reindeer dung	SLE using acetone and isooctane	C18 SPE	HPLC-FD using ABM as internal standard	LLV: 5 µg/kg (ww)	>95	Asbakk *et al.*, (1999)
IVM	Plasma and faeces	SLE using ACN/water (1:1)	C18 SPE	HPLC-FD (quantitation)	LOQ: 0.5 µg/kg	72.4 ±9.3%	Alvinerie *et al.*, (1998)
ABM	Foliage, litter, soil, sediment	SLE using ethyl acetate with Na_2SO_4 to remove excess water	Filtered followed by Florisil SPE, filtered again after derivatisation	HPLC-FD (quantitation)	LOD (µg/kg) LOQ Foliage 0.20 0.60 Litter 0.20 0.60 Soil 0.10 0.30 Sediment 0.10 0.30	Foliage 83 Litter 85.2 Soil 86.7 Sediment 89.6	Sundaram and Curry (1997)
IVM	Cattle dung	SLE using methanol	Alumina SPE	TLC after derivatisation (confirmation)	LOD: 40–10 µg/kg (ww)	N/A: detection only	Floate *et al.*, (1997)
ABM	Hops	SLE using methanol/water after re-hydration of hops	LLE into hexane using sodium sulphate (drying agent), followed by amino-propyl (NH_2) SPE	HPLC-FD (quantitation)	LOD: 2 µg/kg LOQ: 5 µg/kg	73 - 108	Cobin and Johnson (1996)
IVM	Cattle dung	SLE using 30% acetone in water and isooctane	C18 SPE followed by LLE using hexane	HPLC-FD (quantitation)	LOD: 1µg/kg (ww)	89-97	Payne *et al.*, (1995)
IVM	Cattle dung	SLE using methanol	Filtered using PTFE mesh filter (Ø= 13mm, 0.5µm)	HPLC-UV (quantitation)	LOD: 100 µg/kg LOQ: 20 µg/kg	> 90 %	Bernal *et al.*, (1994)
IVM	Cattle dung	Soxhlet/adapted mixer method using methanol or ethyl acetate and DCM	Florisil SPE	HPLC-UV (quantitation)	20 µg/kg	78-5% for 0.1-0.5mg/kg range	Lumaret *et al.*, (1993)
IVM	Cattle dung	SLE using 50% ethanol	LLE using ethyl acetate followed by C18 SPE	HPLC-FD (quantitation)	LOD: ~50 µg/kg (dw)	~95	Sommer *et al.*, (1992)
ABM	Lettuce and cucumber	SLE using ethyl acetate	Silica SPE	HPLC-FD	LOD: 40 µg/kg	76-109%	Vuik (1991)
EPR	Sheep dung	SLE using ACN	C8 SPE	HPLC-FD (quantitation)	LOD: 1.0 µg/kg (ww) LOQ 2.5 µg/kg (ww)	78.8 - 87.7	Erzen *et al.*, (2007)

ABM: Abamectin, DOR: doramectin, EMA: emamectin, IVM: ivermectin, MIL: milbemectin, SLE: solid-liquid extraction, ACN: acetonitrile, DCM: dichloromethane, LLE: liquid-liquid extraction, SPE: solid phase extraction, LOD: limit of detection, LOQ: limit of quantitation, LLV: lowest concentration level for which the method was validated

2.2 Analytical Methods

This section will summarise the considerations for the method optimisation and describe the extraction methods employed by this study for the analysis of avermectin residues in manure, soil, sediment and water and the determination of residues on the HPLC.

2.2.1 Method Development

In developing the analytical methods for this study a number of options for various aspects were explored. The rationale for the selection of some aspects, such as extracting solvent, sample clean-up and HPLC mobile phase are described in Table 2-6. Different extraction solvents and methods, clean-up phases and mobile phases used in the HPLC were considered. Subsequent validation (Section 2.3) was used to select methods which proved fit for the purpose of the analysis used in this project.

2.2.2 Preparation of Standards

The test chemical: ivermectin (analytical grade) was obtained from Sigma, UK. Stock standards of approximately 50 µg/mL were prepared in acetonitrile every three months and stored < -20ºC. Further standards were prepared by diluting the stock standard and stored < 5ºC. All standards were prepared using glass vessels to avoid loss of compound due to sorption and stored in amber glass vessels to minimise photodegradation.

2.2.3 Manure Sample Extraction and Clean-up

Prior to analysis, individual manure samples were thoroughly homogenised using a Foss Tecator blade mixer. Following homogenisation, the moisture content was determined for each individual sample. Approximately 40g of homogenised dung was weighed into a pre-weighed foil boat and the sample heated in an oven at 110 °C until a constant weight was achieved and the weight loss recorded. The moisture contents of the manure samples were calculated as follows:

$$\%\ moisture = 1 - \left(\frac{sample\ dry\ weight}{sample\ wet\ weight} \right) \qquad \text{Equation 2-1}$$

If the moisture content of the homogenised manure sample was below approximately 50%, water was added to the samples to bring the moisture content back up to approximately 80%. Re-hydrated samples were then placed on an end-over-end shaker for 24 hours to allow the manure to absorb the water.

Approximately 4 g (wet weight) of homogenised dung was weighed into 50 mL Teflon centrifuge tubes. Fifteen ml of acetonitrile (HPLC fluorescence grade, Fisher Scientific, UK) was added and the tubes briefly mixed using a vortex mixer (Clifton Cyclone, Nickle Electro Ltd., UK). Tubes were placed on an end-over-end shaker (GSL3040, Germany) for 1 hour and centrifuged at 4000 rpm for 10 minutes (Hermle Z513K, Germany). The supernatant was decanted and stored in amber glass vessels. The samples were re-extracted with a further 15 mL acetonitrile and the supernatants combined. Extracts were stored below -20 °C prior to sample clean up and analysis.

Samples were cleaned up prior to HPLC analysis using Strata 1 g end-capped C18 SPE cartridges (Phenomenex, UK). A 15 mL portion of the extract was diluted with 50 mL mili-Q water. Then 50 µL triethylamine (TEA) was added and the mixture gently mixed. The SPE cartridges were activated with 10 mL acetonitrile and conditioned with 10 mL acetonitrile/water (30:70). The diluted sample was loaded onto the SPE cartridges before the cartridges were washed with 10 mL acetonitrile/water (50:50) and the sample eluted with 10 mL acetonitrile.

Sample eluates were taken to dryness under nitrogen at approximately 30 °C and reconstituted into 200 µL acetonitrile. Samples were derivatised by adding 100 µL of N-methylimidazole solution (1:1 N-methylimidazole/acetonitrile) followed by 150 µL of trifluoroacetic anhydride solution (1:2 trifluoroacetic anhydride/acetonitrile) (DeMontigny *et al.*, 1990).

2.2.4 Sediment Sample Extraction and Clean-up

Prior to extraction, sediment samples were defrosted and excess water drained and discarded. The moisture content for individual sediment samples was determined as for manure (Section 2.2.3). Extraction of ivermectin from sediment was performed using approximately 10 g of homogenised sediment and performed as described for manure.

Samples were cleaned up prior to HPLC analysis using Strata 100 mg C18 SPE cartridges from Phenomenex. Ten mL portions of the extracts were diluted with 35 mL water to which 50 μL triethylamine was added. The SPE cartridges were activated with 2 mL acetonitrile and conditioned with 2 mL acetonitrile/water (30:70). The samples were loaded and the cartridges subsequently washed with 2 mL acetonitrile/water (50:50) and the analyte eluted with 4 mL acetonitrile into glass centrifuge tubes.

2.2.5 Soil Sample Extraction and Clean-up

The moisture content determination methods for soil samples were the same as those described for the manure analysis (Section 2.2.3). The extraction methods were as for the manure samples except using 4 g of soil.

Samples were cleaned up prior to HPLC analysis using Strata 100 mg C18 SPE cartridges from Phenomenex using the same methods as those described for the sediment clean-up.

The SPE cartridges were activated with 2 mL acetonitrile and conditioned with 2 mL acetonitrile/water (30:70). The diluted sample was loaded using polypropylene reservoirs above the cartridges. The cartridges were washed with 2 mL acetonitrile/water (50:50) and eluted with 4 mL acetonitrile into glass centrifuge tubes.

Table 2-6 Rationale for selection of various aspects of the analytical methods used for the extraction of ivermectin

Variable	Options Considered	Selected Option	Rationale
Extraction solvent	- ACN - ACN/ water - Methanol - Methanol/ water	100% ACN	Ivermectin is a highly non-polar molecule and therefore should be sufficiently extracted with a non-polar solvent such as ACN. Extracting solvents such as methanol or a mix with water increases the extraction of more polar interferences. The limitations of using 100% ACN were the co-extraction of too many non-polar interferences. However, SPE clean-up and FD detection following derivatisation of the sample demonstrated clear resolution from other peaks in the sample.
Extraction method	- Shaking - Ultra sonication	Shaking	Sonication, a more efficient extraction method was found to increase the extract volume due to temperature increase, the remedy (by cooling) for which would add further time and effort to the method. Sufficient extraction efficiency was demonstrated using the simpler, shaking method.
SPE	- C18, various sizes - Strata X (polymer)	C18, - 1 g (manure) - 100 mg (soil, sediment)	Although strata X SPE columns would have eliminated the need for SPE activation and conditioning, these columns were prohibitively expensive. Large (1 g) C18 SPE cartridges were selected for the clean-up of the manure samples, as smaller sizes became clogged too easily, slowing down sample loading due to the 'dirty' nature of the manure extracts. Smaller (100 mg) SPE cartridges, requiring smaller activation, conditioning and elution volumes were found to be suitable for analysis of the cleaner soils and sediment extracts.
HPLC mobile phase	- ACN/ water at different ratios - Isocratic - Gradient	ACN and water, varying according to a gradient	ACN and water at varying ratios and concentrations were tested. Good separation from interferences and formation of a good peak shape was found to occur in a timely fashion with a gradient, with increasing proportion of ACN. A period of 100% ACN following the elution of ivermectin encouraged the elution of more polar interferences that may otherwise elute in subsequent runs, thereby interfering with ivermectin elution.

2.2.6 HPLC Method

Chromatographic separation was performed on a Sigma Discovery C18 Column (15 cm x 4.6 nm, 5 μm) and a Phenomenex C18 security guard (4 mm x 3 mm), at 30 °C using an Agilent 1100 series HPLC. The fluorescence detector was set to an excitation wavelength of 365 nm and an emission wavelength of 475 nm. Acetonitrile and water was used for the mobile phase following the gradient outlined in Table 2-7. The flow rate was 1 mL/ minute and the injection volume was 50 μL. The retention time of the ivermectin derivative was approximately 17 minutes.

Table 2-7 Mobile phase composition and gradient

Time	% Water	% Acetonitrile
0	5	95
5	5	95
20	0	100
25	0	100

2.3 Method Validation

Validation was performed on the analytical methods used in the UK field study and the Canadian mesocosm study to confirm that the methods were suitable. The linearity, stability, accuracy, precision, and limits of detection and quantification of the ivermectin methods were validated using the methodology recommended by the VICH guidelines (VICH, 1998b).

2.3.1 Spiking of Samples

In order to optimise the analytical methods and to assess the quality of the analytical methods, the appropriate ivermectin-free matrix was spiked with ivermectin in a minimal amount of solvent, extracted and analysed.

Ideally the analytical method should be assessed using matrix-matched blank samples, e.g. examples of the sample matrix before introduction of ivermectin. In preparation for the UK field study (described in Chapter 3), manure was collected at Askham Bryan Agricultural College from non-ivermectin treated

cattle and soil was collected from the field site prior to the introduction of ivermectin-treated cattle. In preparation for analyses of the samples from the mesocosm study (Chapter 3), sediment was sent from the University of Guelph (Sanderson *et al.*, 2007). However, blank water samples (water collected from the study mesocosms, prior to treatment with ivermectin) were not provided, so a proxy blank matrix was required. Water from the Central Science Laboratory pond was used as an alternative. While this is not ideal, it does provide a similar matrix (pond water with aquatic flora and fauna) that is unlikely to have been contaminated by ivermectin since the site was surrounded by arable farmland and not pastured livestock. Blank samples are important to show that there is not a co-eluting substance (with the same retention time) that affects the measurements of the compound of interest. The samples from the control ponds (which were not treating with ivermectin), did not show any co-eluting peaks, so confirming that ivermectin measurements are reliable.

Ivermectin solutions in solvent were prepared at the appropriate spiking levels and added drop-wise as evenly as possible over the surface of the matrix. The samples were stored without caps at room temperature for half an hour prior to extraction to allow for evaporation of solvent and sorption to the matrix.

2.3.2 Linearity of Calibration

Calibration was performed for every sample run and the linearity of the calibration curve was assessed. The linearity of the method is the 'ability to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample' (VICH, 1998a). The linearity of the calibration was assessed by measuring the peak area produced for each of five standards and calculating the plot slope, y-intercept and the correlation co-efficient of a straight line fitted to the calibration data points.

Calibration curves were only accepted if the R^2 measurement of fit for the straight line was above 0.99 and the intercept was less than one percent of the analyte response in the sample (Green, 1996). For example, Figure 2-2 shows the calibration curve used to quantify samples with peak areas in the range of 200

to 1800, where the intercept represents less than 1% of the lowest sample response.

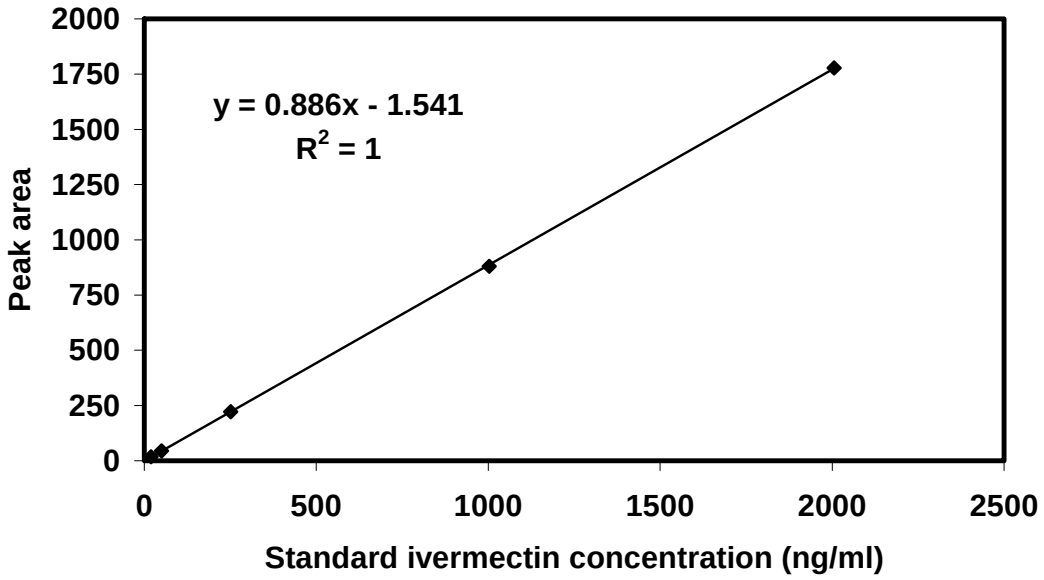


Figure 2-2 Example linearity plot of fluorescence detector response with ivermectin concentration

2.3.3 Stability

Several studies have reported the compromised stability of the ivermectin derivative (e.g. Hernando *et al.*, 2007). It was therefore necessary to assess the stability of derivatised samples awaiting injection onto the HPLC. This was performed by measuring the peak area detected following repeat injections of a derivatised standard and a derivatised sample. Figure 2-3 illustrates the excellent short-term stability of the derivatised standard where the peak areas were not found to vary by more than 2% over 35 hours. However, the sample derivative was not as stable, with a reduction of up to 24% in peak area observed after 5 hours (Table 2-8) and more consistent loss of over 22% after 13 hours (Table 2-9).

Table 2-8 Decline in measured peak area of ivermectin derivative in manure samples after 5 hours

Initial Peak Area	Later Peak Area	% Loss
317.1	239.7	24%
197.4	164.8	17%
180.4	159.4	12%

Table 2-9 Decline in measured peak area of ivermectin derivative in manure samples after 13 hours

Initial Peak Area	Later Peak Area	% Loss
653.8	491.6	25%
303.2	236.6	22%
175.4	133.3	24%

Therefore to minimise the degradation in samples, samples were derivatised immediately prior to analysis, in small batches of up to 6 samples, ensuring derivatised samples were waiting to be analysed for no longer than 3 hours.

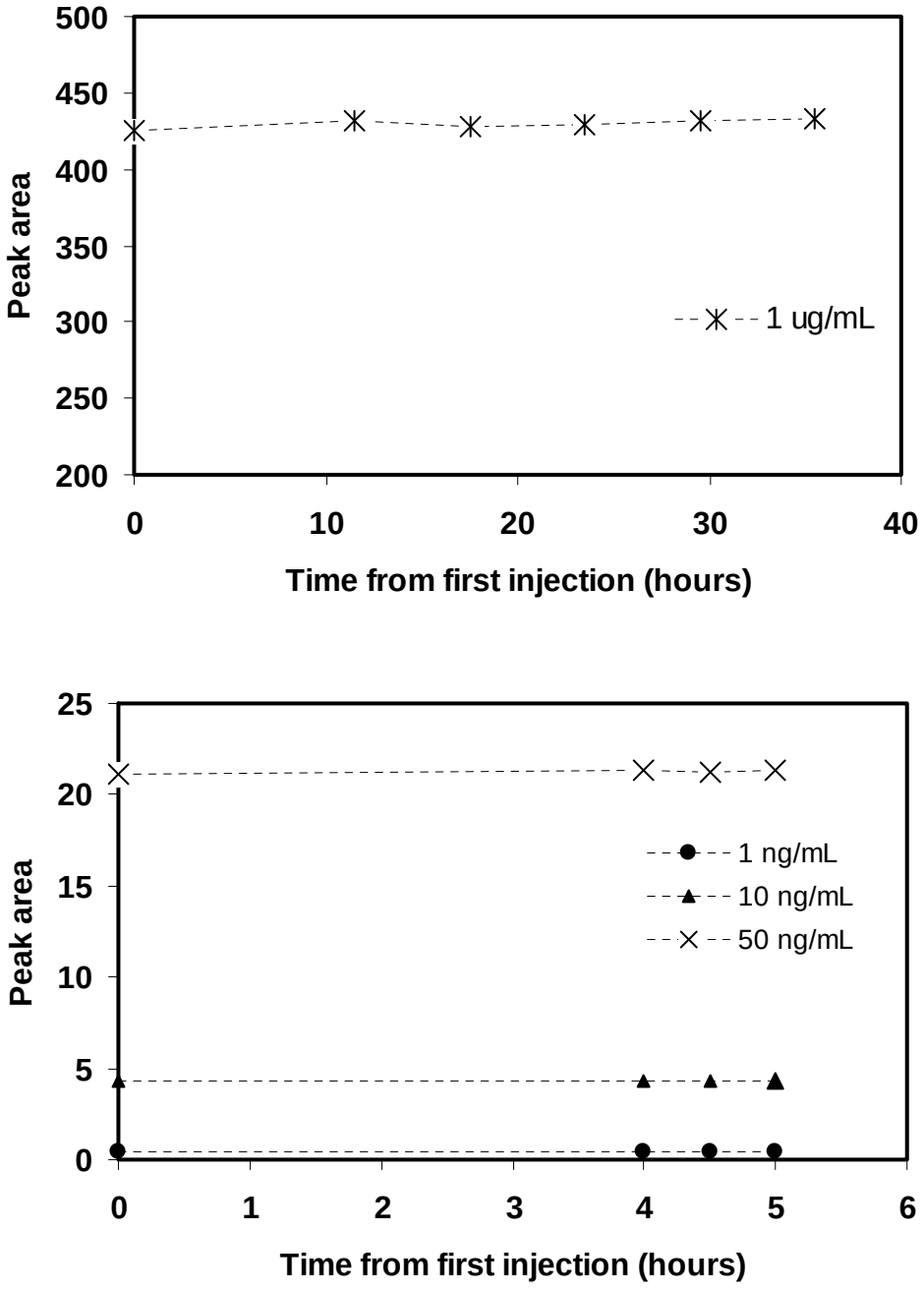


Figure 2-3 Stability of N-methylimidazole derivative during storage at room temperature in standards

2.3.4 Accuracy

Accuracy is defined as 'the closeness of agreement between the value which is acceptable either as a conventional true value or an acceptable reference value and the value found' (VICH, 1998a). In the absence of appropriate reference materials accuracy may be assessed by comparing the amount of analyte detected following extraction, clean-up and analysis of spiked samples. The accuracy of

the extraction and sample preparation methods combined was therefore assessed by the extraction and analysis of blank matrix samples fortified with ivermectin in triplicate, at three concentration levels. The results from these analyses are reported as percentage of recovered compound and are summarised in Table 2-10.

Table 2-10 Mean ivermectin recoveries determined after extraction, sample clean-up and HPLC analysis, concentrations

Sample Matrix	Fortification level	Mean Recovery in % (± standard deviation, n =3)
Manure (mg/kg, dw)	0.09 0.38 1.5	80 ± 10 84 ± 12 84 ± 7
Soil (µg/kg ww)	6 25 125 560	81 ± 24 86 ± 2 88 ± 1 90 ± 4
Sediment (µg/kg ww)	1 10 100	93.1 ± 1.3 90.1 ± 1.4 89.8 ± 1.1

The recovery of ivermectin spiked into manure at environmentally relevant concentrations of 80-84% are acceptable for this study. For soil, the recoveries obtained from the higher spiked concentrations are reasonable (86-90%). However, the lowest spiked concentration is somewhat higher than available measured environmental concentrations, e.g. approximately 1 µg/kg ww, assuming the reported moisture content of 28% water, abamectin in soil (Erzen *et al.*, 2005). In hindsight, the method for analysing soil samples should have been tested at lower concentrations to enable more reliable conclusions to be drawn about the concentrations of ivermectin found in soil samples. However due to time pressures relating to the analysis of the experimental samples, this was not performed. The validation of the analytical methods used for sediment analyses were deliberately performed at elevated concentrations. This method was to be employed in the analysis of samples from a mesocosm study where high concentrations were added to the ponds to monitor dissipation rates. The concentrations measured in the study were the equivalent to approximately 1 to 9 µg/kg ww, within the range of the spiked concentrations. The recoveries obtained for the sediment method (90-93%) are therefore suitable for purpose.

2.3.5 Precision

Precision is defined as 'the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions' (VICH, 1998a). There are three levels of precision assessment; repeatability, intermediate precision and reproducibility. The latter two relate to variation between analysts and laboratories and therefore not relevant in this case.

Repeatability refers to the variation over the same operating conditions over a short period of time. Method repeatability, or intra-assay precision, was assessed by considering the variation in results determined from triplicate analyses (separate extraction, clean-up and detection) at three fortification levels. The results of these tests are summarised in Table 2-11, which shows repeatability in the manure and sediment determinations is adequate. However, determinations in soil at the lowest end of the range (the more environmentally relevant concentrations) are more variable. Measured concentrations in soil within in this range should therefore be reported with caution.

Table 2-11 Precision data for extraction, clean-up and HPLC analysis for each matrix

Sample Matrix	Fortification level	Standard Deviation	RSD (%) or coefficient of variation
Manure (mg/kg dw)	0.09	10	12
	0.38	12	14
	1.5	7	9
Soil (µg/kg ww)	6	24	30
	25	2	2
	125	1	1
	560	4	4
Sediment (µg/kg ww)	1	1.3	1
	10	1.4	2
	100	1.1	1

2.3.6 Limits of Detection and Quantitation

The limits of detection and quantitation are 'the lowest amount of the analyte in a sample which can be detected but not necessarily quantified' and 'the lowest

amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy' (VICH, 1998a).

Table 2-12 shows an example of the limit of detection (LOD) and limit of quantitation (LOQ). In analyses (such as these) where there is a signal baseline, i.e. the signal in the HPLC fluorescence detector from a control sample (sample of the same environmental matrix but excluding the analyte), the signal-to-noise approach is commonly used (VICH, 1998b). The signal measured in a control sample at the location (in the chromatogram) where the analyte is expected to occur is used as the baseline. For the calculation of LOD, this is usually calculated as 3 times the background signal, and the LOQ is 10 times the background signal.

The method limits of detection and quantitation were calculated based on the signal-to-noise approach using Equations 2-2 and 2-3. Since these are calculated based on an analysis of a control sample, e.g. manure without ivermectin, LOQs and LODs were calculated separately for each run.

$$LOD\ (\mu g / mL) = \frac{\left(noise \times 3 \times conc^{n}\ lowest\ std\right)}{Peak\ height\ of\ lowest\ std} \qquad \text{Equation 2-2}$$

$$LOQ\ (\mu g / mL) = \frac{\left(noise \times 10 \times conc^{n}\ lowest\ std\right)}{Peak\ height\ of\ lowest\ std} \qquad \text{Equation 2-3}$$

Table 2-12 Examples of the limits of detection (LOD) and limits of quantitation (LOQ) from this project, following full sample extraction and preparation and quantitation (µg/kg ww)

	LOD	LOQ
Manure	0.8	3
Soil	0.28	0.92
Sediment	0.4	1.3

2.4 Conclusion

A reliable and sensitive analytical method for the quantitation of ivermectin in cattle manure, and sediment has been developed. This is indicated by the low limits of detection and quantitation, good reproducibility, and high recovery of spiked residues (or extraction efficiency) of between 80% and 90% for these matrices, at the concentrations likely to be encountered in the field studies. The validation results in manure in particular, compare favourably to published methods of analyses in similar environmental samples.

However, the methods developed for the quantitation of residues in soil were not so reliable. Measurements at relevant concentrations were variable, with relative standard deviations of approximately 30%. This may be due to substances in the extracted, cleaned-up and derivatised samples co-eluting with the analyte, suggesting the extraction and clean-up methods were not sufficiently selective. Further development of the method for measuring ivermectin concentrations in soil would be recommended, in order to address its reproducibility and at a range of more environmentally relevant concentrations.

3 Fate of Parasiticides in the Pasture

3.1 Introduction

As we have seen in Chapter 1, the initial, lower-tier risk assessments of parasiticides use highly conservative parameters for predicting environmental concentrations. The calculations used to predict a conservative environmental concentration ($PEC_{dung\text{-}initial}$) are based on recommended dosing regimes and default excretion rates for cattle, assuming 100% excretion of the treatment as the parent compound (i.e. no metabolism) in one day. These concentrations are then related to the results of toxicity tests to assess if a risk is posed to the environment.

If risk assessment calculations using these methods find unacceptable risks then the risk assessment needs to be refined, by either refining the effects assessment or by refining the exposure assessment.

When refining the exposure assessment, it is advantageous to gain a better understanding of the inputs of the VMP to the environment and its subsequent fate. Rather than the total residue approach described above, inputs to the field will vary over time according to the treatment regime and the excretion profile of the compound. Once the compound has reached the field we also need a better understanding of its behaviour in the pasture. This will include an assessment of its potential to be transported to other environmental compartments, such as soil, and its persistence in those compartments. This will allow us to focus risk assessment efforts on those compartments most at risk.

This project used Ivermectin, one of the avermectins, as a case-study parasiticide. Ivermectin has been demonstrated to be toxic at environmental concentrations to a range of dung, soil and aquatic fauna with EC_{50} of 0.3 mg/kg (dw), 1.17 mg/kg (dw) and 5.7 ng/L for dung flies, springtails, daphnia respectively (Garric *et al.*, 2007; Jensen *et al.*, 2003; Rombke *et al.*, 2009). However, several aspects relating to the fate of ivermectin under field conditions remain either unknown or

unclear; in particular, there are conflicting reports of avermectin persistence in the environment.

This chapter of the thesis therefore addresses the following aspects of the fate of ivermectin under field conditions: 1) inputs to the field (excretion of residues) 2) persistence in manure and 3) transport to soil; and addresses the consequences which these findings hold for higher-tier risk assessment.

3.1.1 Inputs of Ivermectin to the Field

Parasiticides used in pastured animals enter the environment following excretion from treated livestock. Regardless of the application method, ivermectin tends to be excreted primarily in the faeces, with very low levels detectable in the urine. Metabolism studies have shown 39 – 92% of the applied dose is excreted in the faeces and less than 2% in the urine (Chiu *et al.*, 1990). In addition, ivermectin is largely excreted as the parent compound, with radio-labelled metabolism tests showing the parent drug to account for 39 – 78% of the faecal radioactivity (Chiu *et al.*, 1990).

The application method (and therefore, the applied dose) does, however, determine the quantity of ivermectin reaching the pasture. Several authors have measured ivermectin excretion in cattle following different application methods (Herd *et al.*, 1996; Sommer & Steffansen, 1993; Cook *et al.*, 1996).

Figure 3-1 summarises the excretion data for a range of avermectins where data were expressed in terms of dry weight concentrations or have been converted from the concentrations in wet weights assuming moisture content of 87% (the average moisture content of freshly excreted manure determined in this study). The subcutaneous injections result in maximum concentrations of between 1.1 to 3.9 mg/kg (dw) for ivermectin and slightly lower concentrations reported for abamectin and doramectin, occurring between 3 and 6 days after treatment.

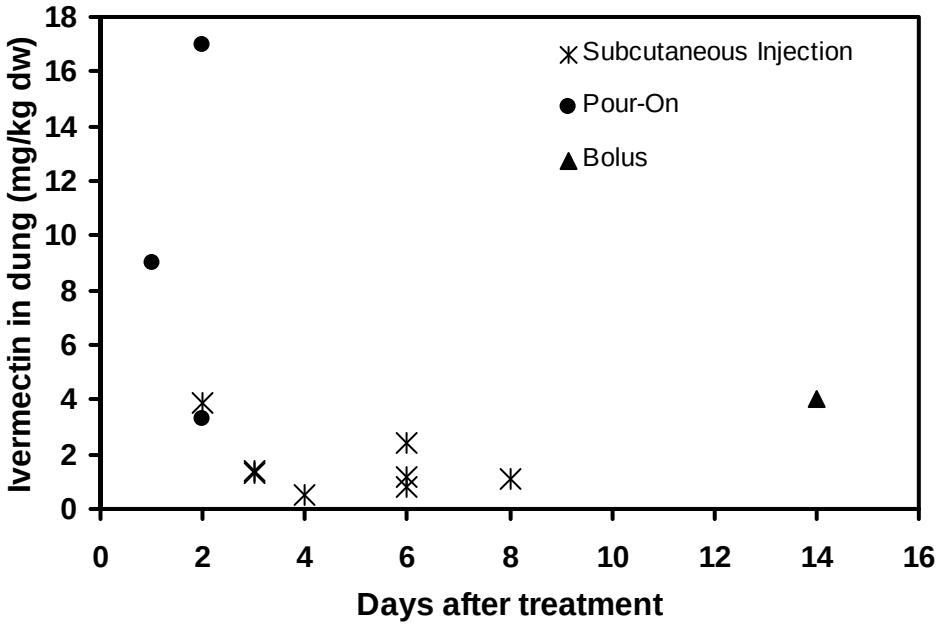


Figure 3-1 Maximum concentrations of ivermectin excreted by cattle and sheep following different application methods, (Erzen *et al.*, 2005; Sommer *et al.*, 1992; Herd *et al.*, 1996; Cook *et al.*, 1996; Lumaret *et al.*, 1993; Lifschitz *et al.*, 2000; Bernal *et al.*, 1994)

Pour-on applications appear to result in the highest and most variable maximum concentrations with concentrations of 9 – 17 mg/kg (dw) reported for ivermectin in cattle, occurring one to two days after treatment. Lafont *et al.*, (2001; 2003) have proved these variable concentrations result from the fact that the major route of absorbance following pour-on application was actually oral, from cattle grooming themselves or each other. Some variation between studies may be attributed to differences in diet with higher levels measured in grain-fed cattle than those grazed on pasture (Cook *et al.*, 1996). The sustained release bolus was designed to slowly release ivermectin to the animal over approximately 100 days (Herd *et al.*, 1996). Following concerns over the long-term damage to dung fauna (e.g. Strong *et al.*, 1996), the sustained release bolus is no longer marketed for use in the UK.

In addition, factors such as treatment regime (e.g. number of times the animals are treated); stocking density and proportion of animals treated will influence field level concentrations. This will be explored via modelling in Chapter 4.

The excretion rate and recommended treatment regimes would be available for industry for performing the environmental risk assessments, from the efficacy and absorption, distribution, metabolism, and excretion (ADME) studies.

3.1.2 Persistence in Manure and Soil

Dung in pasture is sucessionally colonised over time (Hanski, 1991b). This was studied in UK pastures recently by Lee and Wall (2006a), who reported that peak insect abundance was found to occur in three broad but distinct waves, adult coleoptera, followed by adult diptera and then larval coleoptera. Overall, the greatest number of species and individuals occurred 4-7 days after dung deposition (Lee & Wall, 2006a). If parasiticide concentrations degrade rapidly over time then, depending on the organism of interest, use of initial concentrations may over estimate exposure.

Although methods are currently under development for the assessment of controlled, laboratory degradation tests in manure (Committee for Medicinal Products for Veterinary Use, 2009), there is no current regulatory guidance on higher tier approaches to the assessment of parasiticide fate under field conditions. In addition, laboratory fate tests have a number of limitations. These tests tend to spike the matrix under study directly with the compound, ignoring the possible presence of metabolites (which may also be toxic).

The persistence of excreted residues has been investigated more for ivermectin than for any other veterinary medicine. This is likely due to a number of factors. Ivermectin was the first veterinary medicine identified to be excreted at concentrations toxic to dung-degrading fauna (Wall & Strong, 1987). Studies into the potential impact of ivermectin use on pasture ecology were therefore focused on ivermectin and the avermectins. Ivermectin is also one of the most popular veterinary medicines for the treatment of pastured livestock (Boxall *et al.*, 2007). However, the conclusions from the numerous studies into the persistence of ivermectin in the pasture environment are varied. Table 3-1 illustrates the large range of degradation half-lives reported for the avermectin as well as the duration of studies where no degradation was observed. Reported

Table 3-1 Persistence of avermectin residues reported in manure and manure/soil mixtures

Analyte	Matrix	Location & Conditions	DT_{50} (days)	Reference
Abamectin	Sheep faeces/soil	Pasture, Slovenia	23	Erzen *et al.*, (2005)
Abamectin	Sheep faeces pasture	Pasture, Slovenia	30	Erzen *et al.*, (2005)
Doramectin	sheep faeces pasture	Pasture, Slovenia	18	Erzen *et al.*, (2005)
Doramectin	Sheep faeces/soil	Pasture, Slovenia	27	Erzen *et al.*, (2005)
Ivermectin	Fine sandy loam soil	Laboratory	21	Bull *et al.*, (1984)
Ivermectin	Clay soil	Laboratory	42	Bull *et al.*, (1984)
Ivermectin	Coarse sand soil	Laboratory	56	Bull *et al.*, (1984)
Ivermectin	Cattle faeces/soil	Outdoors, summer	7-14	Halley *et al.*, (1989b)
Ivermectin	Cattle faces/soil	Laboratory, dark, 22°C	93- 240	Halley *et al.*, (1989b)
Ivermectin	Cattle faeces	Pasture, Denmark	> 45 days	Sommer & Steffansen (1993)
Ivermectin	Cattle faeces	Pasture, Spain	< 6 days	Lumaret *et al.*, (1993)
Ivermectin	Cattle faeces	Pasture, Argentina, Autumn	> 60 days	Iglesias *et al.*, (2006)
Ivermectin	Cattle faeces	Pasture, Argentina, Late spring	> 180 days	Suarez *et al.*,(2003)

persistence ranges from complete degradation in a field study conducted under drought conditions in Spain (Lumaret *et al.*, 1993), to no observed degradation after 180 days in Argentina (Suarez *et al.*, 2003).

It has been suggested that differences in climatic conditions may explain the differences in the field study results (Halley *et al.*, 1989b). Indeed, temperature can be an important influence of the rate of biodegradation rates of compounds in soil, along with soil type, pH and microbial biomass (e.g. Caceres *et al.*, 2008; Gupta & Ali, 2006). Changes in temperature or moisture are known to affect the degradation rate of VMPs in manure as well. For example, Wang *et al.,* (2006) found an increase in moisture content and temperature to increase the rate of Sulfadimethoxine degradation in manure. However, other investigations of VMP persistence in manure found little influence of temperature (e.g. Winckler & Grafe, 2001). Warmer temperatures may explain the more rapid degradation rates reported in Spain and semi-field laboratory tests in the summer (Lumaret *et al.*, 1993; Halley *et al.*, 1989b). However, in this case, rapid degradation rates may also have been expected in the studies conducted in Denmark in August where no change in concentration was observed after 45 days (Sommer & Steffansen, 1993).

Ivermectin reportedly rapidly photodegrades in water, with a DT_{50} of 0.5 days (Halley *et al.*, 1993). Photodegradation has been suggested as a mechanism for degradation in manure although this is unlikely to be a significant route of degradation in manure as some authors have noted this mechanism would only occur at the surface, in the manure crust (Herd *et al.*, 1996).

The analytical methods used for persistence studies are usually described and presented with reasonable recoveries and limits of detection (Chapter 2). However, recovery tests (the accuracy of the analytical methods) are usually performed following the addition of test compound to freshly excreted manure which may or may not have been frozen prior to use. Dung exposed to field conditions will undergo rapid changes in its physical properties, mediated by biotic and abiotic factors (Lee & Wall, 2006a) (sometimes described as ageing)

which may have the potential to influence the effectiveness of extraction and analytical procedures. It is unclear if these studies described above have taken into account the influence of aging the manure in the field may have on the recovery of the procedure.

3.1.3 Transport and/or Mobility

The exposure of organisms in other environmental compartments such as soil and water will be strongly influenced by the degree to which parasiticides are transported from the manure of treated livestock to the soil and nearby water bodies. The degree to which the parasiticide may be transported will be influenced by the physico-chemical properties of the compound, and the environmental characteristics such as soil type and hydrology, and the activity of soil and dung fauna. The physico-chemical characteristics of three avermectins including ivermectin were presented in Table 1-9. The low vapour pressure of ivermectin indicates it is a non-volatile compound, and therefore unlikely to enter the atmosphere (Bloom & Matheson, 1993). The low water solubility, and a log K_{ow} of 3.21 indicates it is unlikely to be transported in the aqueous phase (Bloom & Matheson, 1993).

Laboratory soil and manure sorption/desorption tests give an indication of ivermectin's ability to distribute between soil or manure and water. The K_d of 227 – 333 for ivermectin and 15,600 for closely related doramectin in soil indicate strong sorption to soil and manure. Compounds with distribution coefficients expressed on the basis of organic carbon in soil (K_{oc}) values higher than 4,000 are generally regarded as non mobile (Hollis, 1991). The high K_{oc} of 12,600 – 15,700 together with low water solubility of ivermectin indicates a tendency to sorb strongly to organic matter (Halley *et al.*, 1989a). This suggests residues will not easily be leached from the organic rich manure. Halley *at al.,* (1989a) confirmed the low potential of ivermectin to leach from manure with a series soil column studies with ivermectin-spiked manure and manure from treated cattle. Following application of tritium-labelled ivermectin to soil columns, 10 – 48% of the applied radioactivity was recovered in the leachate, but ivermectin was not detected (however, the limit of detection is unclear, reported

as 13 ± 7 ng ivermectin only). Further studies simulating run-off from a 93 m^2 farmyard with the manure from five treated cattle did not detect ivermectin in the run-off water (Nessel *et al.*, 1989). However, the limit of detection in this study was fairly high, at 0.01 µg/L, higher than the EC_{50} for daphnia of 0.0057 µg/L (Garric *et al.*, 2007). The report did state that the toxicity of the run-off was assayed for ivermectin using Daphnia and no ivermectin related toxicity was observed (Nessel *et al.*, 1989).

These laboratory and semi-field studies confirm the low potential of ivermectin residues to be leached from treated manure. Residues are likely to remain firmly bound to the organic matter in manure. However, these results are dependent on the limit of detection of the analytical methods. Note that ivermectin is toxic to aquatic fauna at concentrations approaching and below the limit of detection of the methods employed by the studies above (Garric *et al.*, 2007).

There is also the potential for particulate-bound ivermectin to be transported to the soil beneath manure via the transport of manure particles. These particles could enter the soil by either preferential flow (channels or pores in the soil) or by the incorporation of soil and dung fauna such as earthworms. Although there have been several studies investigating the degradation of ivermectin residues in soil, there is limited monitoring data describing the actual occurrence of residues in the soil following exposure through manure in the cattle pasture scenario. In the few available studies, Erzen *et al.,* (2005) reported abamectin concentrations of up to 1.4 µg/kg (dw) in a pasture used by abamectin-treated sheep and an Environment Agency monitoring study tested sites used by ivermectin treated cattle and pigs and detected ivermectin in the soil at concentrations of 46 µg/kg at the pig farm only (Boxall *et al.*, 2006a). Currently, there are no studies investigating the transport of ivermectin residues from the manure of treated cattle in the pasture environment. Investigations into the potential of residue transport in the pasture scenario would ideally take place under field conditions where the dung can be naturally colonised by dung (and soil) fauna, an important mechanism for the incorporation of manure into the soil.

3.1.4 Dissipation in Aquatic Systems

Ivermectin may enter aquatic water bodies such as streams and ponds in the pasture, either by transport of ivermectin bound particulate matter or by direct defecation into the stream. On reaching the water the compound has the potential to degrade in the water, partition to the sediment, persist or degrade in the sediment.

Under laboratory tests using clean water, ivermectin, abamectin and doramectin were found to rapidly photodegrade with half-lives of 4.5 hours to 12 hours (see Table 3-2). Under more realistic scenarios, using artificial pond water or the presence of sediment, the avermectins appear more persistent in water, with DT_{50}s of four days reported for abamectin in artificial pond water Wislocki *et al.,* (1989) and DT_{50}s of three days for ivermectin in a laboratory water/sediment test (Loffler *et al.*, 2005).

Table 3-2 Dissipation rates for avermectins in water, [1]Abamectin, [2]Ivermectin, [3]Doramectin

Matrix, test conditions	DT_{50}	Reference
Water, 'summer' conditions	0.5 days[1]	Halley *et al.,* (1993)
Water	4.5 hours[3]	
Water	12 hours [1]	Wislocki *et al.,* (1989)
Artificial pond water	4 days[1]	
Pond sediment	2-4 weeks[1]	
Water, laboratory water/sediment system	2.9 ± 0.4[2]	Loffler *et al.,* (2005)
Sediment, laboratory water/sediment system	>100 days	

If the avermectins reach surface waters they are likely to adsorb to the sediment in ponds due to their high affinity to organic matter. In their soil/sediment systems, Loffler *et al.,* (2005) found 16 – 42% of the applied ivermectin was rapidly sorbed to the sediment. This was attributed to the highly lipophilic nature of ivermectin. It was also suggested that additional interactions in the sediment were likely, such as the formation of adducts with cations. The degradation of ivermectin in the sediment was too slow to be measured within the study, although some degradation in the sediment appears to have occurred within the 100 day study period. The authors classify ivermectin as moderately persistent, and suggest that if residues do reach water bodies, accumulation in natural

sediments is likely, especially in anaerobic sediments with high organic matter content (Loffler & Ternes, 2003). Even under these more realistic conditions, e.g. using artificial pond water instead of pure water, or the presence of sediment, these studies may still not reflect the fate of residues in the field environment where concentrations may be influenced by the presence of insects and plants.

3.2 Aims and Hypotheses of the field studies

Although there is existing data in the published literature describing ivermectin excretion, manure from treated animals is required for the fate investigations. It therefore made sense for completeness to replicate an excretion rate study. It also gave the opportunity to identify metabolites excreted alongside the parent compound. The first hypotheses are therefore simple:

- The rate of ivermectin excretion from subcutaneously treated cattle in a UK pasture environment can be confirmed
- Known ivermectin metabolites can be positively identified in excreted manure

As discussed in Section 3.1.3, measurement of the degradation of ivermectin residues under controlled laboratory conditions may not be representative of the situation in the field. Ideally, persistence should be assessed in the presence of representative pasture organisms and using manure containing naturally excreted residues. There is literature available on avermectin persistence under field conditions but there are large discrepancies in rates of degradation. In addition, the case-study scenario employed by this project is the impact of parasiticide treatment in the UK pasture environment for which data does not currently exist. This yields a further hypothesis:

- Ivermectin residues do not degrade under field conditions in a UK pasture environment

Published laboratory studies that have demonstrated that avermectin residues are unlikely to be transported in the aqueous phase given the measured degradation

of residues in soil. However, there are limited data on available on the transport of residues excreted by pastured livestock to the soil environment, and no data available for the pastured cattle scenario explored in this project.

- Ivermectin residues are transported into the soil following deposition of dung from treated cattle.

Finally, small scale assessments of the fate of ivermectin in aquatic systems have been undertaken under controlled, standardised laboratory tests. However, there is little information available on dissipation under field conditions, in the presence of pond biota.

- The rate of ivermectin dissipation in water bodies may be assessed under semi-field conditions

3.3 Methods

A field study was conducted between July and September 2006 at Askham Bryan agricultural college, near York. A separate semi-field study was conducted at the University of Guelph, Canada by Hans Sanderson, from which water and sediment samples were taken and sent to York for analysis and assessment of fate in an aquatic system.

This section will describe the methods employed to assess the ivermectin input from treated cattle, the effect of moisture content on extraction efficiency, the persistence of residues in field aged manure, movement to soil and dissipation in water.

3.3.1 UK Study Site and Cattle Treatment

The studies took place in an area of permanent pasture owned by Askham Bryan Agricultural College, situated south-west of York. These pastures were used by the college for grazing of their non-lactating dairy cattle, which had not previously been treated with ivermectin.

The farmer treated 30 approximately 1 year old cattle with a subcutaneous injection of Virbamec (an ivermectin preparation trade name) Injectable Solution for Cattle using the recommended dose rate of 200 µg per kg body weight on 6th July 2006. The Virbamec instructions recommend treating cattle 3, 8 and 13 weeks after cattle have been turned out to pasture. The single treatment used in this study, administered on the 6th July, would correspond to the 3rd treatment in a standard treatment regime (NOAH, 2008).

Cattle were treated in the field and remained in the field for the duration of the study. The rate of ivermectin excretion following treatment was monitored in freshly voided dung over 18 days following application. In addition, the persistence and possible dissipation of ivermectin in the field was investigated by monitoring over time concentrations in cowpats and in the soil layers directly beneath.

The physico-chemical properties of the pasture soil used in this study are listed in Table 3-3:

Table 3-3 Physico-chemical characterisation of the field study soil

Characteristic	Value
pH (suspended 2:5 in water)	6.3
Organic matter oxidisable (%)	2.65
Clay (%) D < 0.002 mm	11.9
Silt (%) 0.002 < D < 0.02 mm	13.3
Silt (%) 0.02 < D < 0.05 mm	6.0
Sand (%) 0.05 < D < 2 mm	68.8
Cation Exchange Capacity (meq per 100 g)	9.6
N_{total} (%) dw	0.16
P (mg/kg) dw	49
K (mg/kg) dw	56
Mg (mg/kg) dw	169
Ca (mg/kg) dw	1191
Na (mg/kg) dw	46
Cu (mg/kg) dw	27
Fe (Ext. EDTA) (mg/kg) dw	852
Zn (mg/kg) dw	58
Mean Colony-forming units per g dry soil (SD)	1.8E+06 (2.6E+06)

In addition, daily rainfall records for the test period were obtained from Askham Bryan Agricultural College, measured at the college's weather station.

3.3.2 Excretion Rate

Freshly excreted dung from treated cattle was sampled at intervals of 1, 2, 3, 6, 9, 13 and 18 days post treatment to determine the rate of ivermectin excretion following a subcutaneous injection of ivermectin. On each sampling day the cattle were observed and when one defecated approximately 250 ml of fresh manure was collected. The ear tag identification number of the individual cow was recorded. This procedure was repeated until at least four samples had been collected from 5 – 6 different cattle. Unfortunately, only four replicates are available for day 3 following an accident in the laboratory.

Two aliquots were taken immediately from each individual sample: one of approximately 4 g was taken to determine ivermectin concentration and stored below 20 °C prior to sample clean-up and analysis and the second aliquot, approximately 50 g, was taken and then dried to a constant weight in an oven at 110 °C to determine moisture content.

3.3.3 Metabolite Identification

A selection of extracted and cleaned-up manure samples, from both freshly excreted and field-aged manure were analysed using tandem mass spectrometry by Thermo Fisher Scientific using the LTQ Orbitrap. Accurate mass MS and targeted MS/MS experiments were performed. Analysis was performed in the positive mode with ivermectin and ivermectin-metabolites detected as sodium adducts. Separation was performed on a Hypersil Gold C18 column (50 x 2.1 mm, 1.9 µm particle size). The mobile phase composition was 60% formic acid (0.1%) and 40% acetonitrile for the first minute with the proportion of acetonitrile increasing to 100% at 10 minutes (see Table 3-4). The mobile phase was maintained at 100% acetonitrile for another 5 minutes before falling back to 40%. The flow rate was 0.2 mL/min and the injection volume was 5 µL.

Table 3-4 Mobile phase gradient used for LTQ Orbitrap analysis

Time (min)	% Formic acid (0.1%)	% Acetonitrile
0	60	40
1	60	40
10	0	100
15	0	100
15.1	60	40
18	60	40

The samples were screened for the presence of a number of possible metabolites identified in the literature, shown in Figure 3-2.

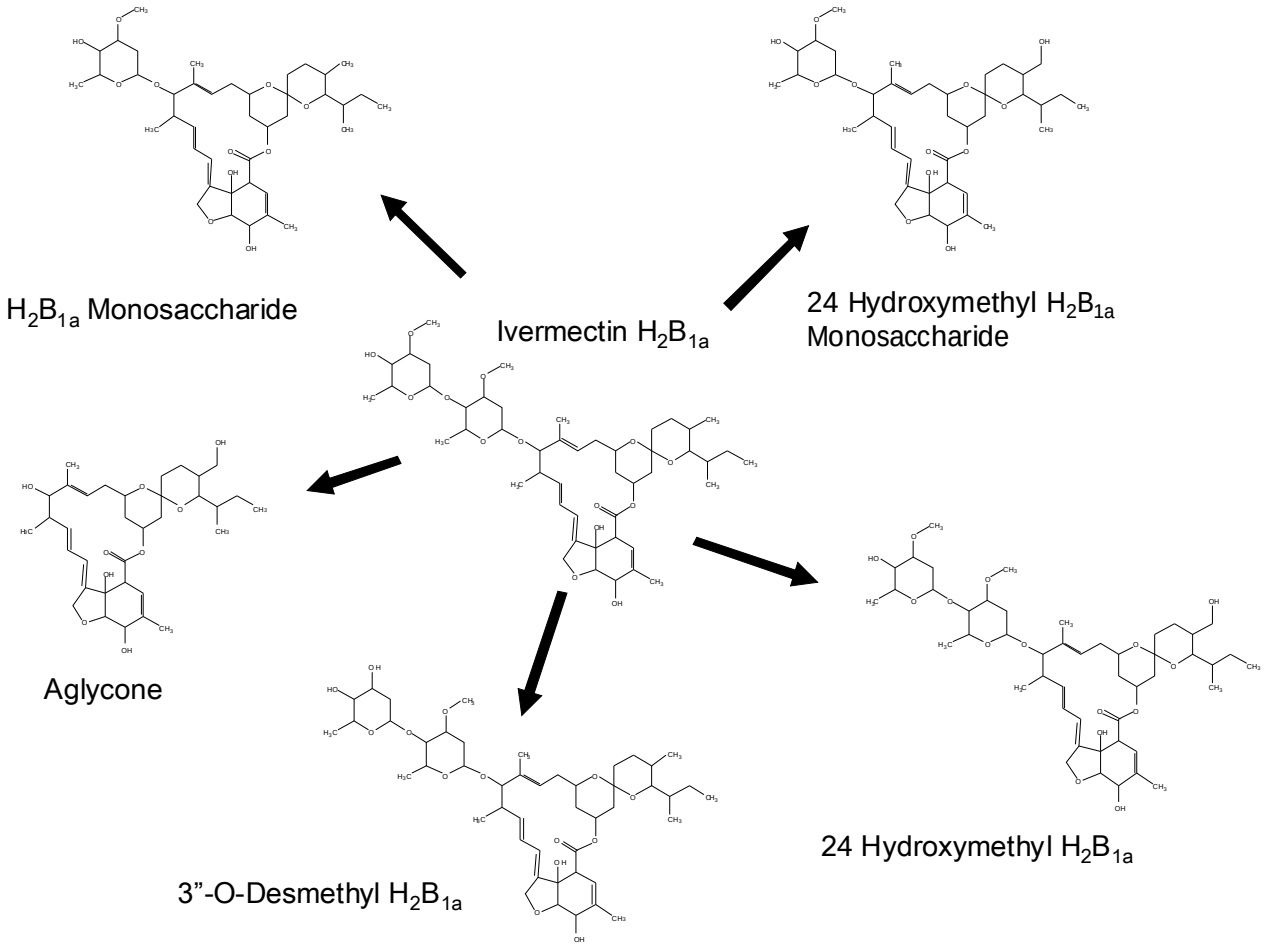


Figure 3-2 Ivermectin metabolites identified from literature, Miwa *et al.*, (1982)

3.3.4 Dissipation of Manure Residues

A separate study was designed to monitor ivermectin persistence in the pasture environment. The aim was to record how ivermectin concentrations in cow pats change over time as the pats are exposed to realistic field conditions. To avoid unintended side-effects of sub-sampling pats in the field, it was decided that removing the entire pat from the field for sampling in the laboratory would be more accurate. Since this sampling procedure is a destructive process (the entire pat was removed from the field) it was necessary to use artificial cow pats. The use of artificial pats ensures that the degradation data between pats sampled on different days is consistent. Artificial pats were created by collecting enough dung on a single day to construct all the artificial pats, homogenising this dung and then creating new, artificial pats which were exposed to the pasture environment. Since each pat is created from the homogenised mixture, they all have the same initial ivermectin concentration and moisture content. Each artificial pat was the same size and experienced the same field and weather conditions, so that all the pats collected on a single day can be considered as true replicates.

Freshly voided dung was collected on the 3rd day after treatment, when high ivermectin concentrations were expected after this treatment type (Herd *et al.*, 1996). The dung was thoroughly homogenised using a plaster mixer attached to an electric drill. Four samples of the homogenised material were then collected and stored to determine the concentration at the start of the study and to check the homogeneity of the dung.

An area of pasture approximately 85 m^2 was set aside to be used for the persistence study. This area was protected from cattle trampling using an electric fence. The grass in this area was cut to approximately 10 centimetres a few days prior to the start of the study and a grid was set out and marked using labelled stakes. At each sample position approximately 500 ml of the homogenised dung was used to construct an artificial cowpat, which was placed on a piece of nylon netting to facilitate removal during sampling. The mesh size of the netting was

approximately 5 mm in an attempt to allow most invertebrates access from the soil below.

Dung for the control pats was collected the day before ivermectin treatment and stored below 5 °C until the rest of the dung was collected on the third day after treatment. These control pats were added to the grid at the same time as the others, and experienced the same weather conditions as the pats from the treated animals. Pats from treated and untreated (control) cattle were randomly assigned positions on the grid. On each sampling occasion, occurring after 3, 7, 10, 19 and 37 days of field exposure, one entire pat selected at random, was removed for each replicate. A further sampling time point was scheduled for day 71, but by that time, all remaining pats had disappeared from the sampling area.

In addition, after the cow pat and netting was removed, the soil beneath was sampled at three depths, 0-1, 1-2 and 3-5 cm. A section of the soil directly adjacent to the location of the pat was removed to allow the soil directly beneath the pat to be sampled at different depths, see Figure 3-3. Soil samples were stored at -20 °C prior to analysis. These samples were taken immediately following pat sampling, i.e. on days 3, 7, 10, 19 and 37. Despite the absence of pats on day 71, a soil sample was still taken on that day.

Figure 3-3 Soil sampling directly beneath the pat.

The choice of sampling time points was initially based on a projected scenario where the degradation occurred very quickly. Early time points were chosen close together to ensure that even in the event of fast degradation, enough data would be collected. Later time points were chosen once the early time points had been analysed to allow good coverage of the degradation profile, once some indication was available of the nature of the profile. In addition, this allowed the possibility of collecting more replicates at each time point in the eventuality that the initial data suffered from high variability. In actuality, the variability was sufficiently low to not require extra replicates.

3.3.5 Dissipation in Water and Sediment: Mesocosms

A semi-field aquatic mesocosm study was performed to explore the fate of ivermectin in surface waters and to explore effects on the aquatic community (Sanderson *et al.*, 2007). The experimental work was designed and conducted by Hans Sanderson and his colleagues at the University of Guelph with fate analysis performed as part of this study. The aspects of the study relating to the fate of ivermectin and performed as part of this thesis will be reported here.

The study was conducted during the summer and autumn of 2004 at the university of Guelph mesocosm facility, Ontario, Canada. The mesocosms ponds were approximately 1.2 m deep (water depth of 1 m), 3.9 m diameter, 11.95 m^2 surface area and have a volume of 12,000 L. Approximately 50% of the mesocosm bottom is covered with 5 cm depth of sediment. Ivermectin was applied at nominal concentrations of 30, 100, 300 and 1,000 ng/L. These concentrations were selected by the University of Guelph and it should be noted that these are elevated concentrations and therefore are unlikely to occur in the environment under normal livestock practices.

There were three replicate ponds at each concentration and three positive control ponds, into which the co-solvent, acetone was applied. Treatment occurred over 4 days. Water and sediment samples were taken from the start of the study, including the period of application by Hans Sanderson and his team. Composite water samples (4 L) were taken following treatment using a 2.5 m long depth-

integrated water sampler from which a subsample of 250 ml was taken from ponds treated at 1,000 and 300 ng/L and 300 ml was taken from ponds treated at 100 and 30 ng/L and control ponds. The water samples were extracted using HLB SPE, eluted with dichloromethane, the solvent evaporated and the residues reconstituted into 1 ml acetonitrile. A 100 µL aliquot was shipped to York for derivatisation and analysis. Composite sediment samples were taken from three locations in each mesocosm from which a subsample of 75 mL was taken. The sediment samples were frozen on the day of sampling before being shipped to York for extraction and analysis.

The dissipation half-live ($DT_{50aqueous}$) of ivermectin in the water phase of the mesocosms was calculated for the four treatment levels assuming first order kinetics using the software and guidelines recommended by the FOCUS work group on degradation kinetics (http://viso.jrc.it/focus/dk/). The concentrations determined in the three mesocosms of each treatment level were used as the three replicates in the data analysis.

In addition, the fate of ivermectin was examined in more detail. A model that considers single first-order degradation in the water and sediment phases and exchange between the two phases was fitted to the water and sediment data simultaneously. Since the dissipation behaviour of ivermectin at the four treatment levels was similar, this model was only applied to a single treatment level. One mesocosm from the treatment level of 1,000 ng/L was modelled. This mesocosm was chosen as it was from the treatment level which exhibited the least variation between replicate ponds and therefore best fit the expected concentration decline. The kinetic model was fitted to the data using the ModelMaker software (version 4.0) with the least squares method used to identify the model parameters that best fitted the data (Figure 3-4). Concentrations were converted to percentages of the applied dose (over 4 days), assuming a sediment depth of 5 cm and a bulk density of 0.8 g/cm^3. However, in one case the sum of ivermectin in the water and sediment was over 100% of the theoretically applied dose (132%). This is probably due to the variability in concentrations across the depth of the sediment.

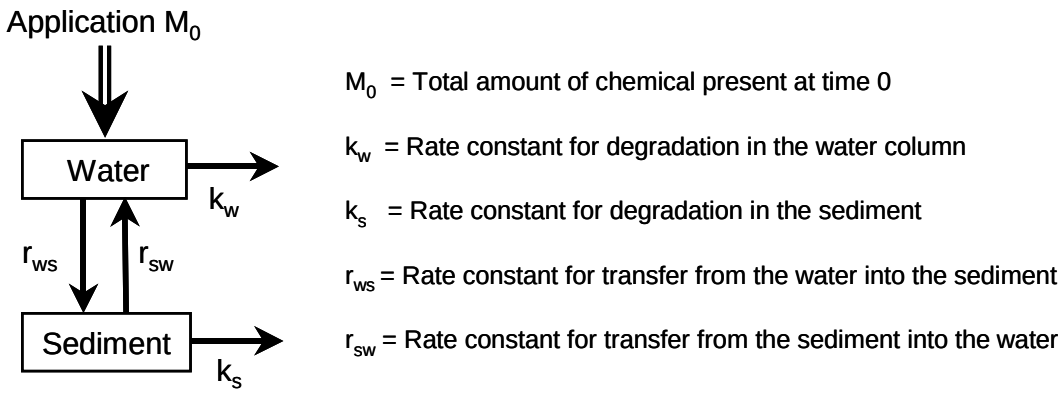


Figure 3-4 Model considering dissipation in water and sediment compartments and degradation in water

3.4 Results

This section will report the results attained from the fate studies: the ivermectin excretion rate; the metabolites identified; the effect moisture content has upon extraction efficiency; the dissipation and persistence of residues in the pasture; and dissipation of ivermectin in aquatic mesocosms.

3.4.1 Excretion Rate

The excretion study data were found to resemble a positively skewed Poisson distribution, which, following square root transformation, approximated a normal distribution (Anderson-Darling $P > 0.05$). As there were different numbers of replicates in different time-point groups, the data were analysed by Generalised Linear Model (GLM). Statistically significant differences were detected in the concentrations measured between time-points ($P<0.005$). Post hoc Tukey tests (Table 3-5) indicated there were highly significant differences between most time-points up until day 9.

Table 3-5 Post hoc Tukey test results following GLM analysis of excretion data, *= P<0.05, **= P<0.005

Day	1	2	3	6	9	13	18
1	---		**				
2		---			*	**	**
3			---	**	**	**	**
6				---			*
9					---		
13						---	
18							---

The results of the ivermectin excretion study are presented in Figure 3-5. These results show that ivermectin concentrations in excreted manure increased rapidly for three days, reaching peak levels of approximately 1.3 mg/kg (dry weight). After three days concentrations fell with an approximate half-life of 3.2 days. At the end of the study, 18 days after treatment, ivermectin excretion levels were at approximately 0.1 mg/kg (dry weight).

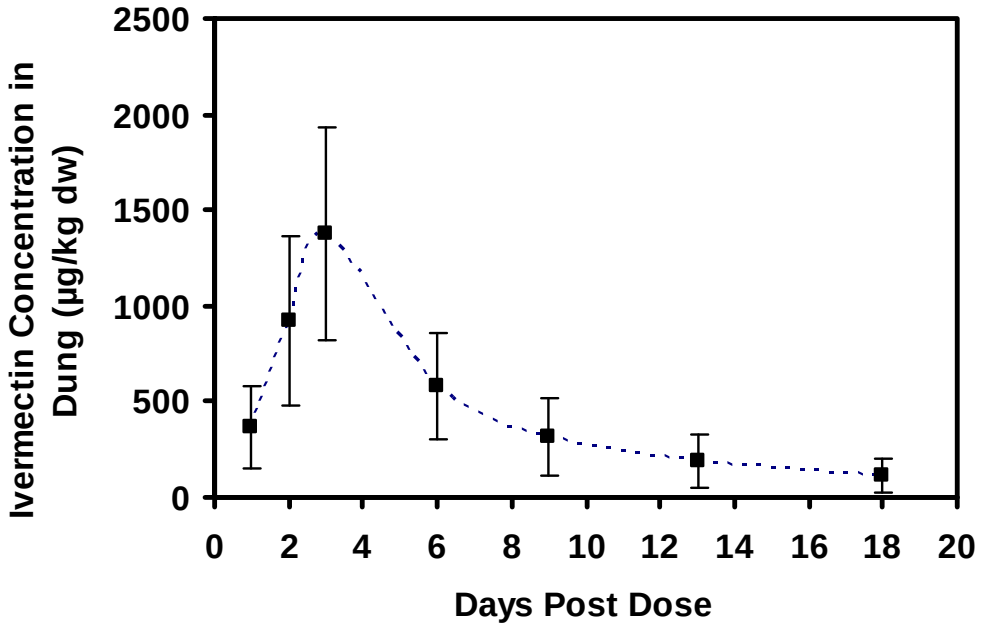


Figure 3-5 Excretion of ivermectin following subcutaneous injection, with 95% confidence limit error bars

3.4.2 Metabolite Identification

Chromatograms produced following HPLC-FD analysis showed two peaks in addition to ivermectin consistently visible in the dung from treated cattle that

were not present in the 'control' pats. These peaks were clearly visible in the chromatograms of the freshly excreted as well as the field-aged pats (Figure 3-6). It was hypothesised that these peaks were ivermectin metabolites formed in the cattle.

A selection of samples were analysed using an Orbitrap, in order to determine the identity of these two metabolites. Figure 3-7 illustrates the high sensitivity and mass resolution achieved in the Orbitrap analysis, with the carbon isotopes pattern of ivermectin clearly shown.

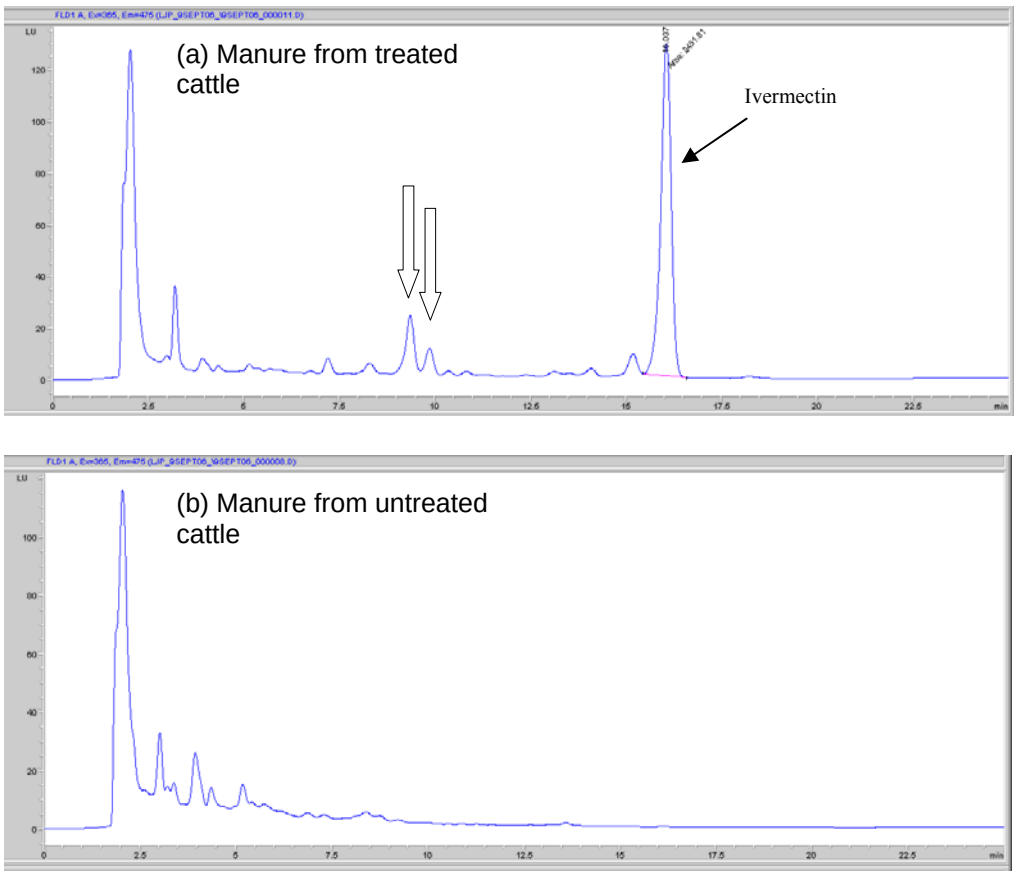


Figure 3-6 HPLC-FD chromatograms of manure from (a) ivermectin treated and (b) untreated cattle, both samples have been exposed to field conditions for 10 days. Two unidentified peaks eluting at approximately 9 and 10 minutes, were consistently visible in samples from treated cattle

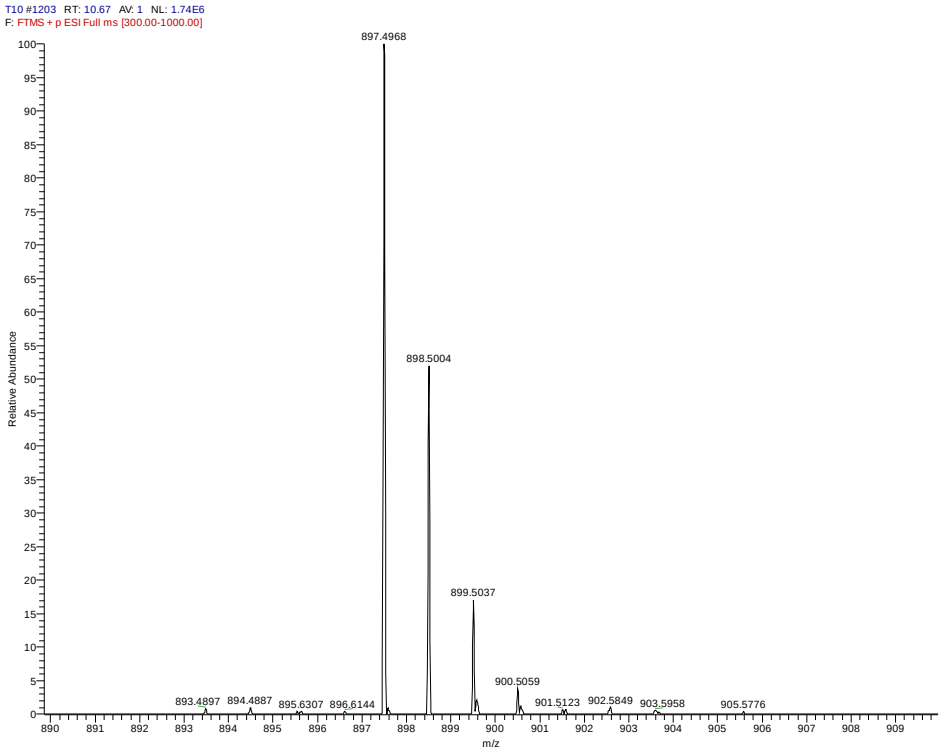


Figure 3-7 Accurate mass full MS of ivermectin, from 0.5 µg/mL standard in acetonitrile

Two metabolites were positively identified in the samples. The adduct ions with mass to charge ratios predicted from the literature were fragmented to give MSMS confirmation of their identity (Figure 3-8). This structural information confirmed the identity of one of the metabolites as 24-Hydroxymethyl H_2B_{1a}. Figure 3-8 shows the mass shift in the fragmentation pattern equivalent to the loss of one oxygen, the only difference between the parent compound and this metabolite.

Ivermectin and the 24-Hydroxymethyl H_2B_{1a} metabolite showed the characteristic neutral loss of 144, the mass of one sugar from the molecule. The samples were screened to look for this common fragment and an ion with a mass to charge ratio of 883.4814 was identified. The structural information attained from fragmentation confirmed the identity of a second metabolite as 3"-O-Desmethyl H_2B_{1a}. The structures of these two metabolites are shown in Figure 3-9.

In the absence of standards for these two metabolites, the concentrations of the two analytes are reported in Figure 3-10 as ivermectin equivalents. These

ivermectin equivalents were obtained by determining the relative size of the peak areas for the metabolites compared to the peak area for ivermectin from the same chromatogram. The pattern of excretion of the two possible metabolites roughly follows that of ivermectin with concentrations of approximately 10-50% (metabolite 1) and 20-60% (metabolite 2) of ivermectin for up to about eight days after treatment.

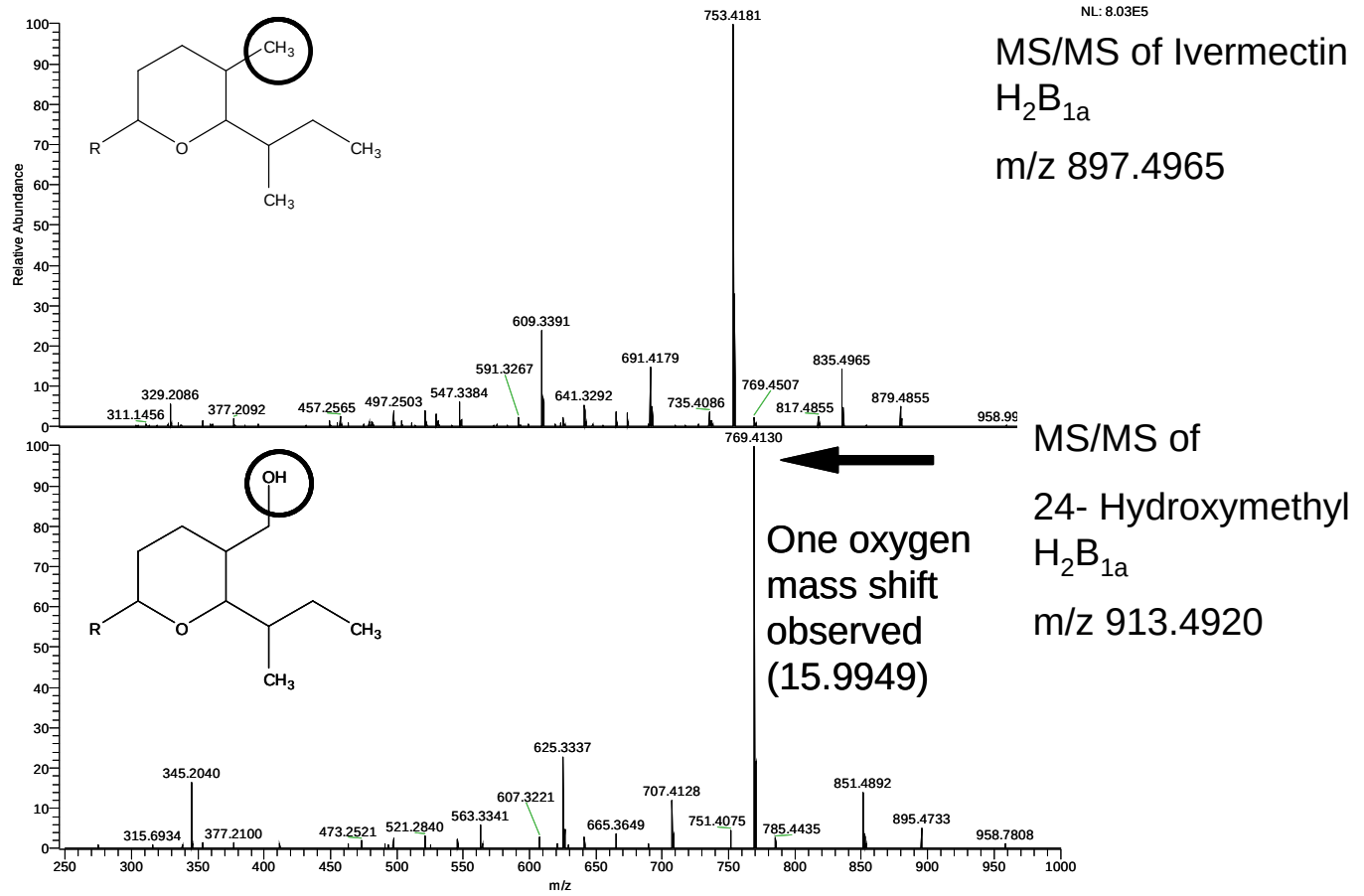


Figure 3-8 Accurate mass MSMS of the ivermectin (top), and the 913.4920 (bottom) adducts, showing the mass shift in the fragmentation pattern equivalent to the loss of one oxygen

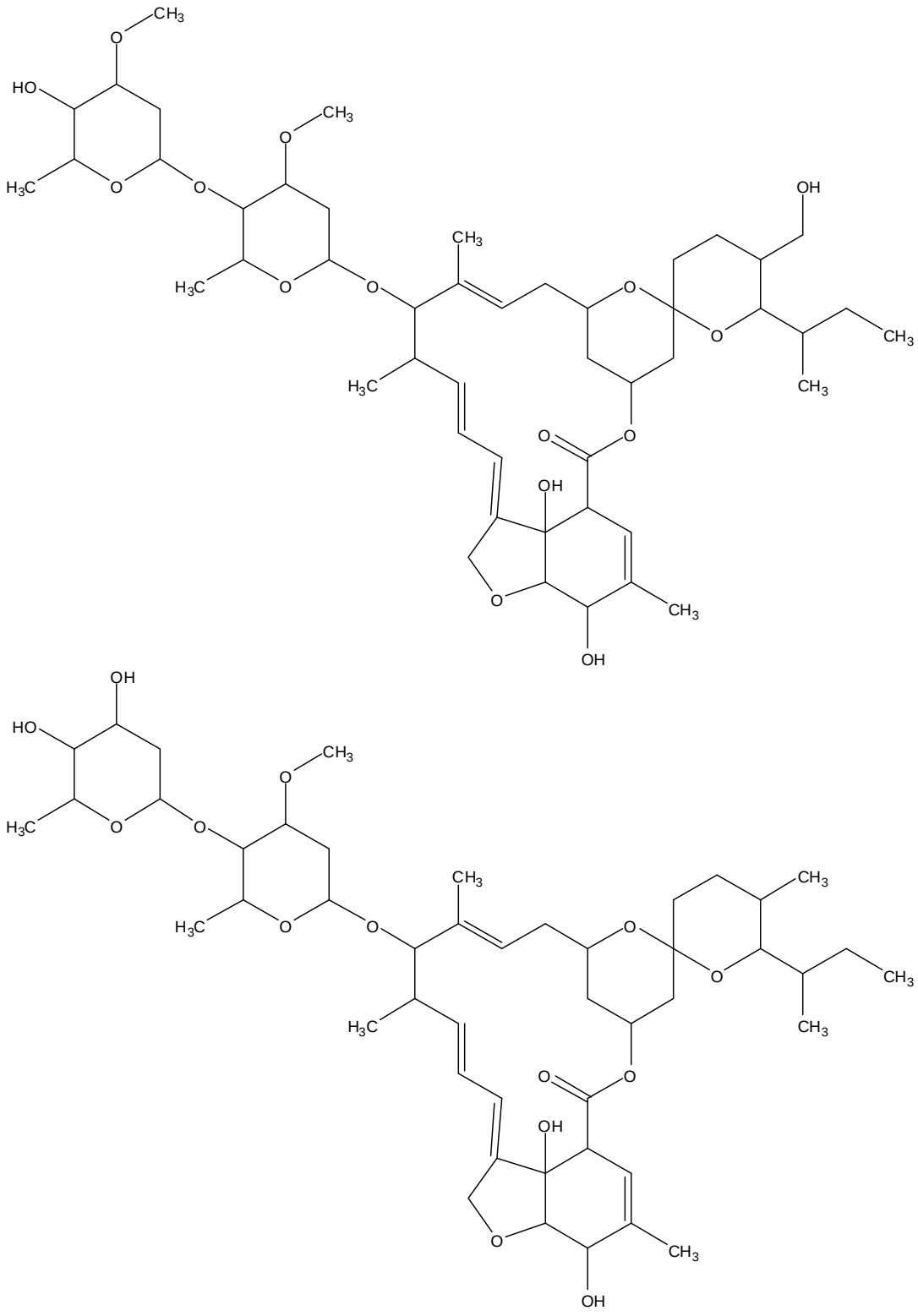


Figure 3-9 Structure of the two metabolites identified in the manure samples, 24 Hydroxymethyl H2B1a (top) and 3”-O-Desmethyl H2B1a (bottom)

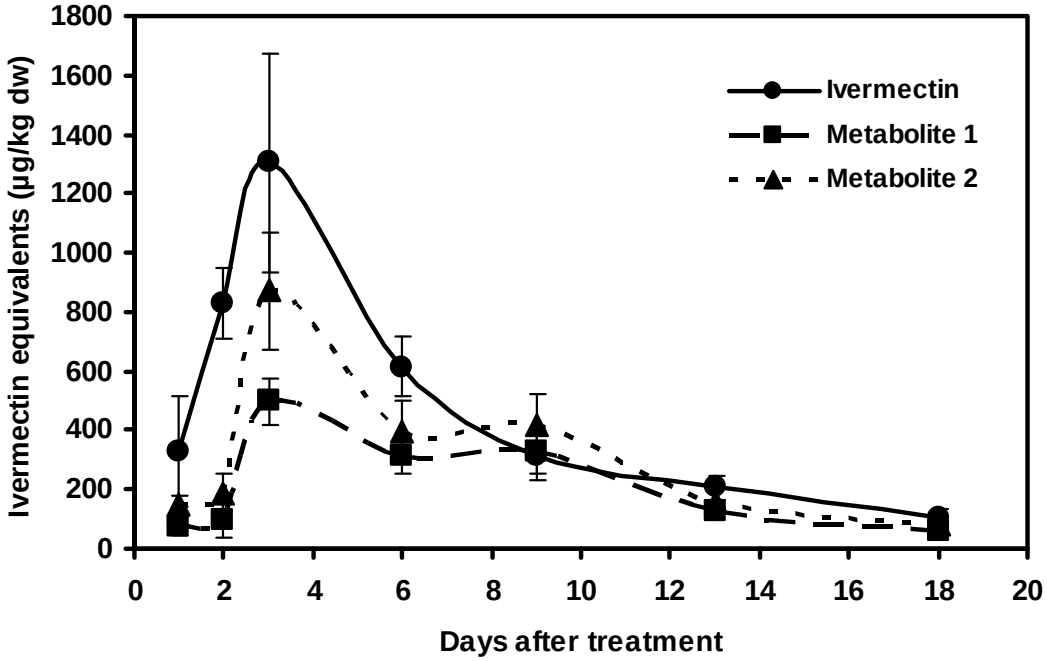


Figure 3-10 Excretion of ivermectin metabolites, with standard error bars

3.4.3 Dissipation of Manure Residues

Manure from treated cattle was sampled after exposure to pasture conditions in the field for different periods of time. Ivermectin concentrations measured after 0, 3, 7, 10 and 37 days were fairly similar, at around 1.3 mg/kg (Figure 3-11). However, the concentrations measured in samples collected after 19 days in the field were over 6 times lower, with a mean of approximately 0.2 mg/kg. The measurement of higher ivermectin levels in the manure sampled 37 days after field exposure indicated the lower levels in the day 19 samples were unlikely to be due to degradation of residues in the field.

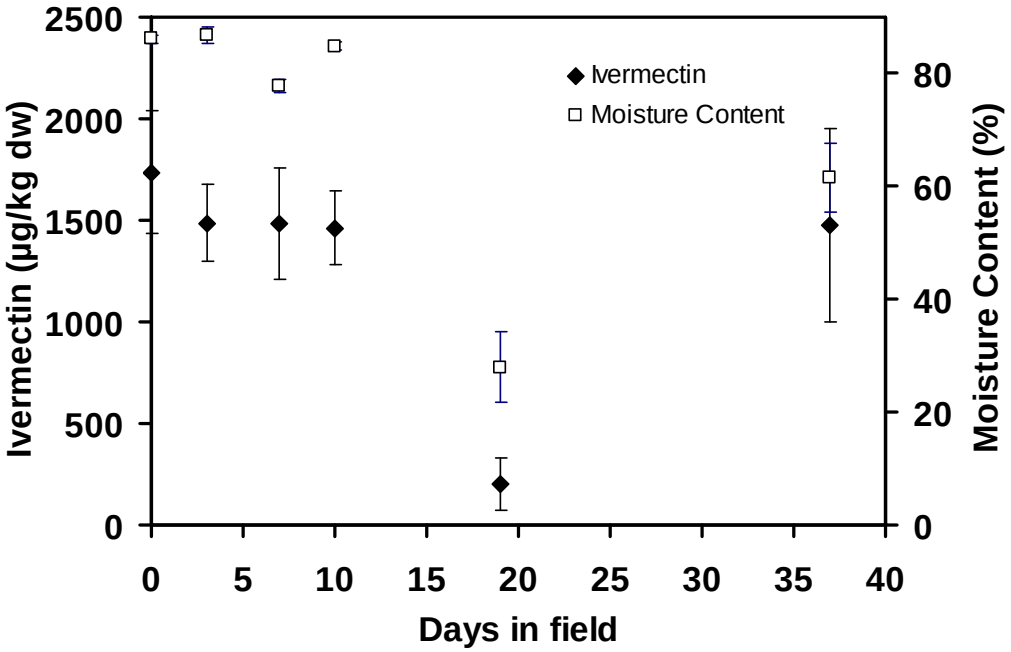


Figure 3-11 Initial determination of mean ivermectin concentrations and mean moisture contents for field aged samples with 95% confidence limits

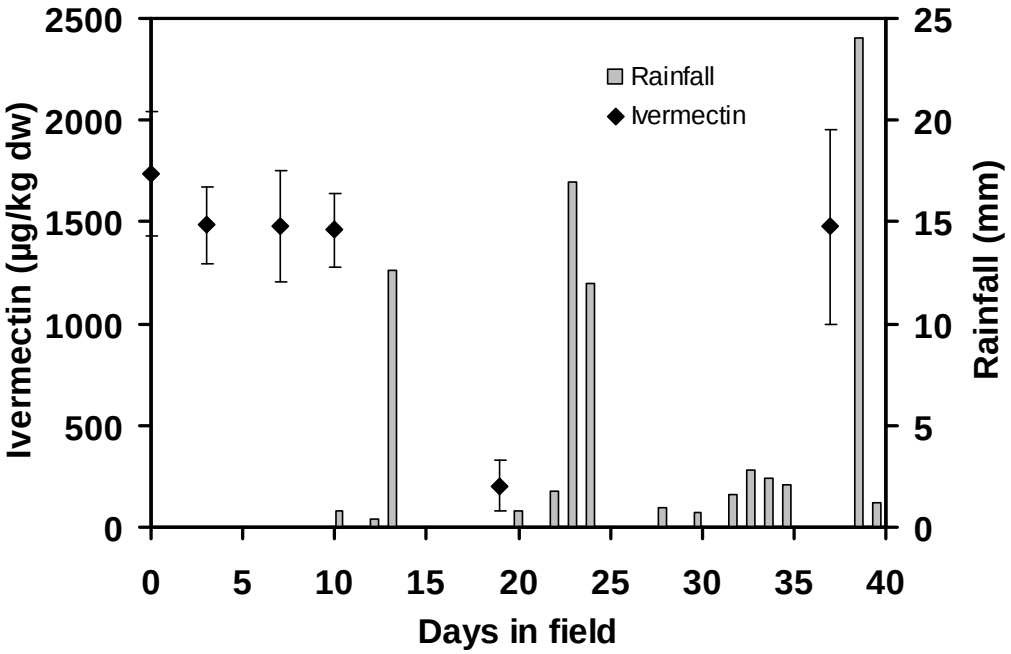


Figure 3-12 Initial determination of mean ivermectin concentrations and mean moisture contents for field aged samples together with daily rainfall

When the results are considered alongside the moisture contents determined for the same samples it was apparent the manure samples with the lowest moisture contents also exhibited the lowest ivermectin measurements. Figure 3-12 displays the initial measured concentrations alongside the daily rainfall measured at Askham Bryan Agricultural College. The anomalous result on day 19 follows six days without rain, whereas the next sample on day 37 follows only two dry days. Despite little rainfall at the beginning of the experiment, the pats themselves initially have high moisture content and take several days to dry out.

A new study was designed to explore the effect of moisture content on ivermectin extraction efficiency (see Section 3.5) and following the results of this study the samples collected after 19 days in the field were re-hydrated to the same moisture content of samples collected on day 0. The water was added and then the samples were placed on a shaker overnight to allow the water to be reabsorbed by the dry manure.

After re-hydration the samples were re-extracted and re-analysed. Figure 3-13 presents the revised ivermectin concentrations determined following exposure to field conditions. Re-hydrating the homogenised, field-aged manure prior to analysis yielded a seven-fold increase in measured concentration on day 19. The high variability exhibited by the samples taken after 37 days in the field corresponds to highly variable moisture content. These data indicate there was no significant change ($P > 0.05$) in the concentration of ivermectin throughout the 40 days of the field study.

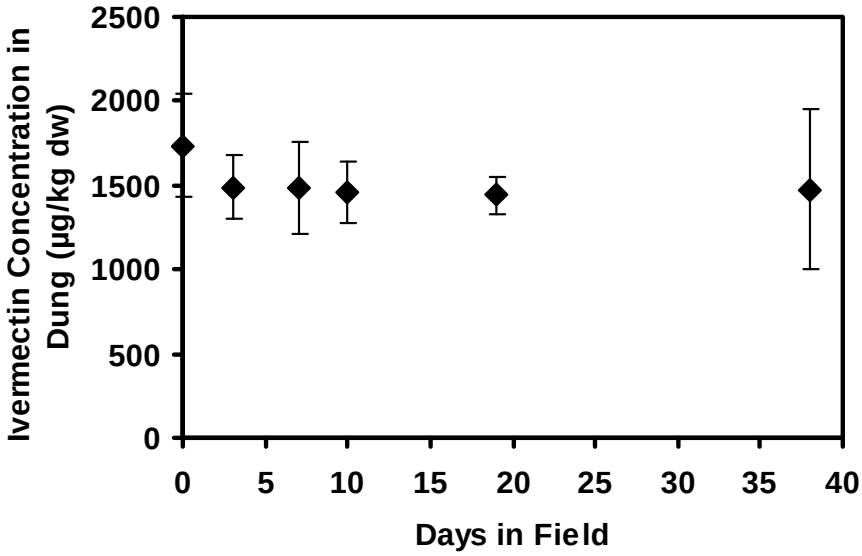


Figure 3-13 Ivermectin concentrations determined in field aged samples following re-wetting of the samples and repeat analysis of samples at day 19, with 95% confidence limits

3.4.4 Transport of Residues from Manure to Soil

To investigate the transport of ivermectin residues from manure to soil, the soil directly beneath the replicate cow-pats at different depths was analysed for ivermectin residues. The ivermectin concentrations measured in the soil are summarised in Figure 3-14.

After seven days of exposure in the field, ivermectin residues were detected in the top cm of the soil only, for three of the four sample sites, with a maximum concentration of 2.89 µg/kg (ww). After 10 days of exposure, residues were detected in only the top 1 cm, at two of the four sites tested. After 19 days, residues of up to 1.63 µg/kg were detected in the top cm in only two of the five sites. After 38 days, residues were detected at every site in the top cm and in four of the five sites in the 1-3 cm layer, with peaks of 2.46 and 0.77 µg/kg respectively.

By day 71 the cow-pats were no longer visible, only the netting with grass growing through remained. On this occasion, only the soil was sampled. Residue levels were significantly higher, present in samples from every site (5 out of 5), reaching peak levels of 91.44 and 19.89 µg/kg in the 0-1 and 1-3 cm

layers respectively. The mean concentration in the top layer of soil was 57 µg/kg.

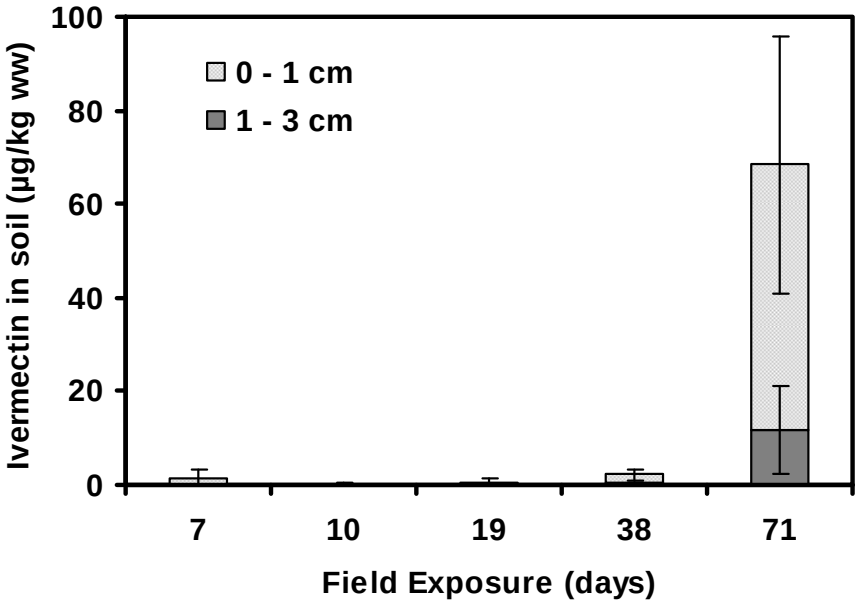


Figure 3-14 Ivermectin concentrations measured in the soil direct beneath pats from treated cattle

3.4.5 Dissipation in Water and Sediment: Mesocosms

Water samples were taken from the ponds at each treatment level (1,000, 300, 100, 30 ng/L) and the control ponds were analysed. Figure 3-15 shows the dissipation rates calculated for ivermectin in the water phase: the sum of partitioning to the sediment and degradation in the water. The ponds with the lowest treatment levels of 100 and 30 ng/L exhibited the most variable results. The fit of the dissipation curve for these two treatment levels were the least robust. The DT_{50} calculated using these two treatment levels (of 3.1 and 5.3 days) are therefore the least reliable. However, the fit for the dissipation graphs using the data from the highest treatment levels were fairly robust. The dissipation half-lives calculated for these two ponds were similar, at 4.1 and 4.3 days.

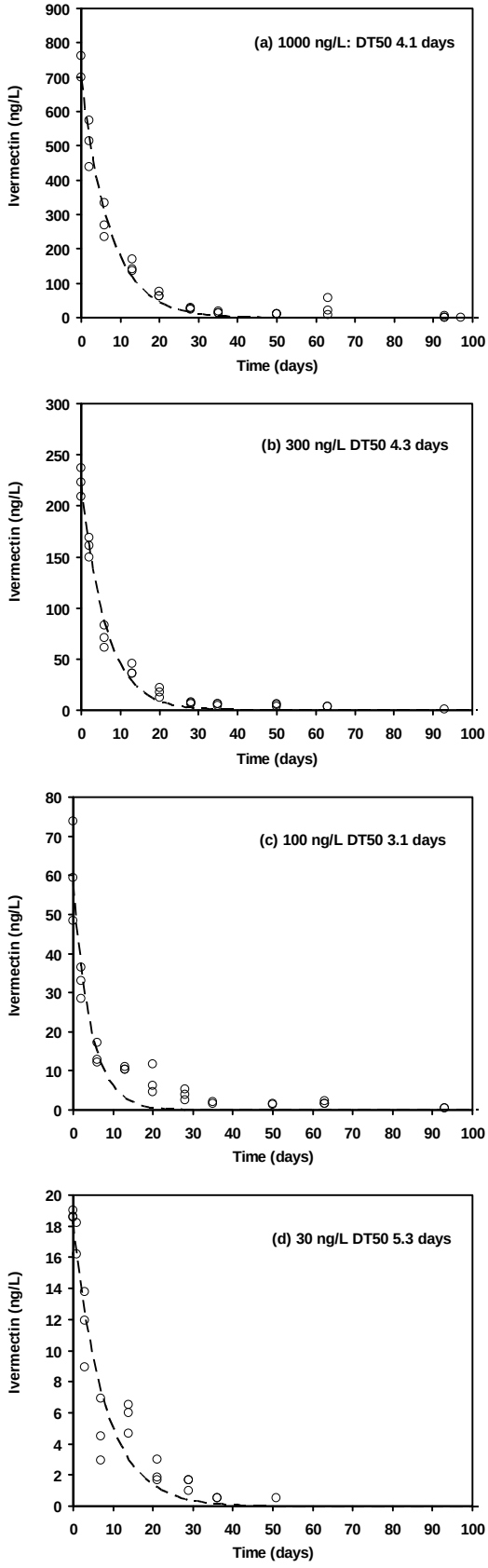


Figure 3-15 Dissipation rates calculated for ivermectin in the water phase (sum of partitioning to sediment and degradation) for the four treatment levels

The modelled dissipation of ivermectin concentrations in the sediment and water phases (and exchange between the two phases) of one of the ponds treated at 1 µg/L is shown in Figure 3-16. A single pond from the highest treatment level was selected for modelling, to provide an indication of the dissipation of ivermectin in the water-sediment system. The concentration in the sediment reached a maximum of 29.8 µg/kg (dw) 54 days after application, after which the concentration did not noticeably decline. This concentration represents approximately 70% of the ivermectin applied to the pond.

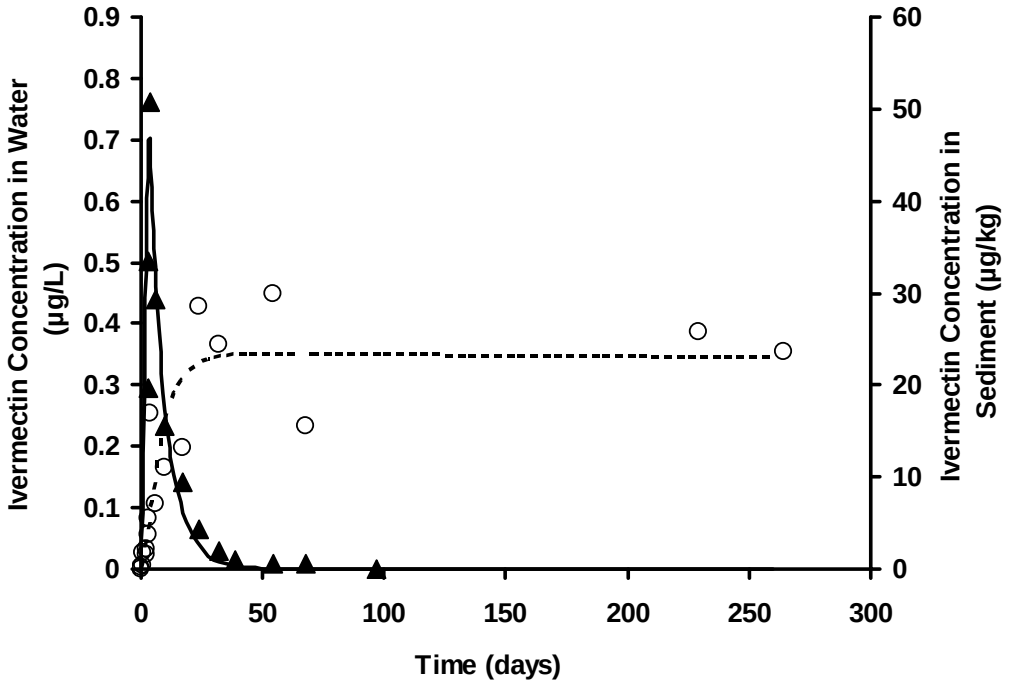


Figure 3-16 Measured and modelled ivermectin concentrations in the water (filled triangles and black line) and sediment (open circles and dashed line) in mesocosm treated with 1µg/L

Ivermectin rapidly partitioned from the water compartment of the mesocosm to the sediment. Modelling of the water-sediment system demonstrated that the loss in the water compartment could not be explained by partitioning to the sediment alone (Table 3-6). There is likely to have been some degradation in the water phase, probably due to photolysis. Ivermectin was shown to be highly persistent in the sediment with little or no partitioning back to the water phase (r_{sw}). Dissipation was not discernible in the sediment; hence a DT_{50} sediment was not calculated. A build-up of ivermectin over the first 3-4 weeks following treatment

was observed, which then stabilized between 20 and 30 µg/kg for the highest treatment level (shown in Figure 3-16).

Table 3-6 Water - sediment model parameters, a=Fixed values

k Sediment (per day)	0.0001[a]
k Water (per day)	0.062
r Sediment→ water (per day)	0.0001[a]
r Water→ sediment (per day)	0.078

3.5 Effect of Moisture Content

The results shown in Figure 3-11 above suggest that the moisture content of the dung samples was affecting the extraction efficiency of ivermectin. This was particularly evident in the dung sampled on day 37, where re-hydration of the pats following several days' rain had increased the apparent ivermectin concentration above that sampled on day 19.

3.5.1 Hypotheses

Following from these anomalous results, two hypotheses were proposed:

- Extraction efficiency for ivermectin is dependent upon the moisture content of the sample
- The effect of moisture content on extraction efficiency can be reversed by re-hydrating the samples

3.5.2 Methods

To assess the effect that moisture content had on the analysis of ivermectin residues a laboratory test was performed using spiked 'control' manure. Approximately 4 g of manure (collected prior to ivermectin application) with previously determined moisture contents was accurately weighed into PTFE centrifuge tubes. 1.2 µg ivermectin was added evenly, drop-wise over the surface of the manure in the tubes. Three tubes were immediately capped to prevent any loss of moisture and the manure in the other tubes was slowly dried

under a gentle stream of nitrogen. Ivermectin was unlikely to be lost due to volatilisation during the drying presence due to its low volatility (vapour pressure of $< 2 \times 10^{-7}$ Pa). Tubes were capped at different intervals to prevent further evaporation and weighed in order to calculate their new moisture content. The manure samples were extracted with 2 x 15 mL of acetonitrile as previously described in Chapter 2 but without the addition of water. The extracts were cleaned-up using SPE and analysed by HPLC-FD as described in Chapter 2.

In addition, one set of manure samples collected in the dissipation study was extracted and analysed with and without the addition of water. These samples were from the group with the lowest moisture content (approximately 30% water), collected after 19 days of exposure in the field.

In addition to the main dissipation study, a separate field study conducted in collaboration with colleagues from ECT was conducted between 16th May and 11th July 2006 (which shall be referred to as the ECT field study). The only contribution of this ECT field study to this thesis is the residue analysis in dung, uncorrected for the affect of moisture content on extraction efficiency. This study was aimed at investigating the impact of ivermectin treated cattle manure on pasture fauna. The aim was to have dung samples simultaneously exposed in the field containing different initial ivermectin concentrations, for the purpose of generating dose-response ecotoxicity data. In brief, cattle were split into groups of four and treated with ivermectin on different days. Cattle were treated 13, 7, 4, 3 and 2 days prior to the start of the field study. One group of cattle remained untreated. On the 16th May, manure from each of the six groups was collected over the course of one day, thoroughly homogenised and stored < 5°C overnight. An area approximately 85 m^2 was sectioned off from the main pasture, the grass trimmed and the protected from trampling by cattle with an electric fence. On the 16th May, artificial pats from each group were placed in the field as described above for the persistence field study. These pats were removed following 2, 7, 14, 28 and 56 days of exposure. An aliquot of approximately 20 g was set aside for ivermectin and moisture content analysis and the remaining manure sent to colleagues at ECT, Germany, for fauna analysis. Prior to extraction, wet manure

samples were homogenised using a Foss Tecator and drier samples were homogenised using a pestle and mortar. Approximately 4 g was used for residue analysis and the remaining manure was using to determine the moisture content. Although the results of the faunal analysis were unavailable for inclusion in this thesis, the results of ivermectin residue analysis have been presented here and used to support the effects of the moisture content hypothesis.

3.5.3 Results

The results from the laboratory study on the effect of moisture content on extraction efficiency are shown in Figure 3-17. There is a strong, positive correlation between sample water content and the proportion of the applied ivermectin that was extracted. This relationship was modelled using non-linear regression to fit a 4-parameter logistic curve to the data using least squares. This effect appears to diminish approaching approximately 40- 50% water content. After this threshold, the moisture content does not appear to influence extraction efficiency.

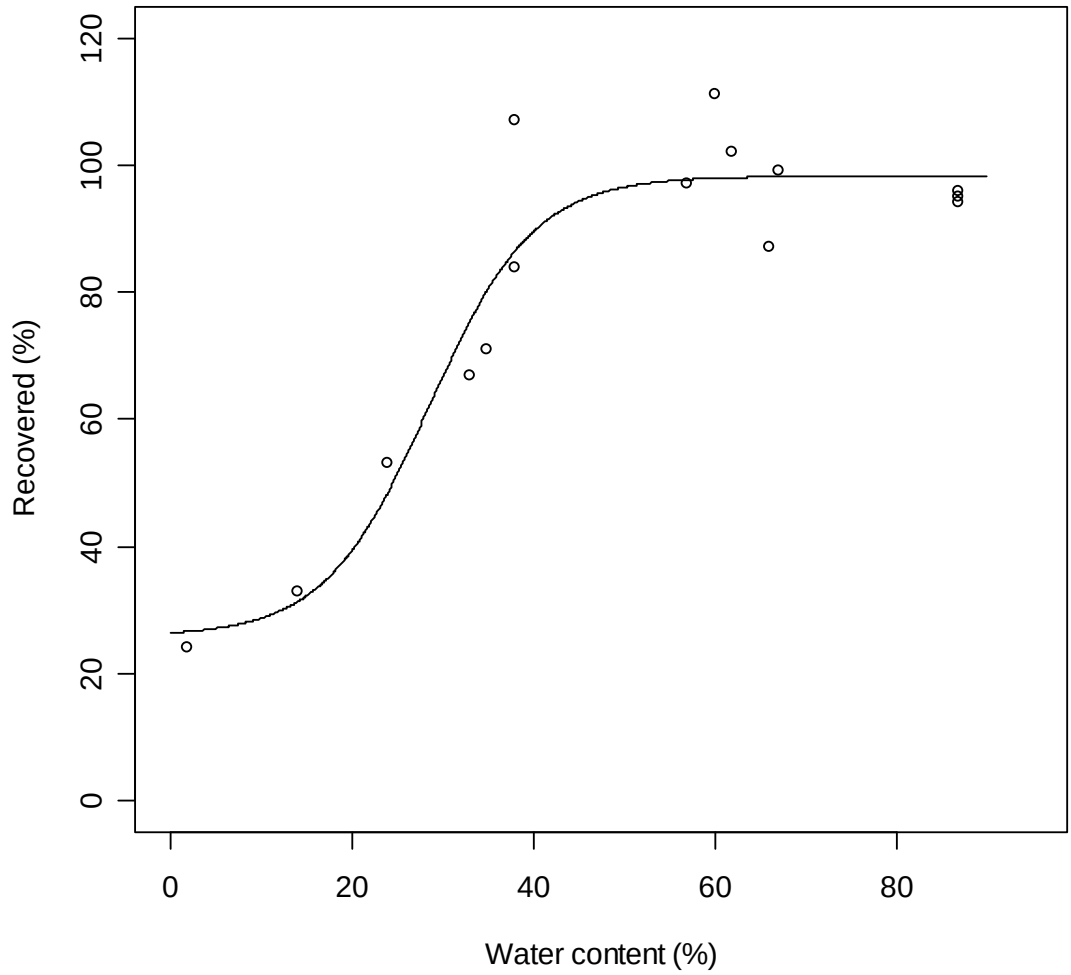


Figure 3-17 Extraction efficiency (% of spiked ivermectin extracted) of ivermectin in manure samples dried under nitrogen

Manure samples from the field study conducted with ECT were extracted and analysed before the effect of sample water content on extraction efficiency was known. Unfortunately, there was insufficient sample remaining to repeat the extraction and analysis with re-hydrated manure. The measured ivermectin concentration as a percentage of the initial ivermectin concentration in the study samples were calculated and are shown plotted against sample water content in Figure 3-18, combined with the results from the persistence field study. Again, the strong positive threshold between water content and the proportion of initial ivermectin extracted is evident.

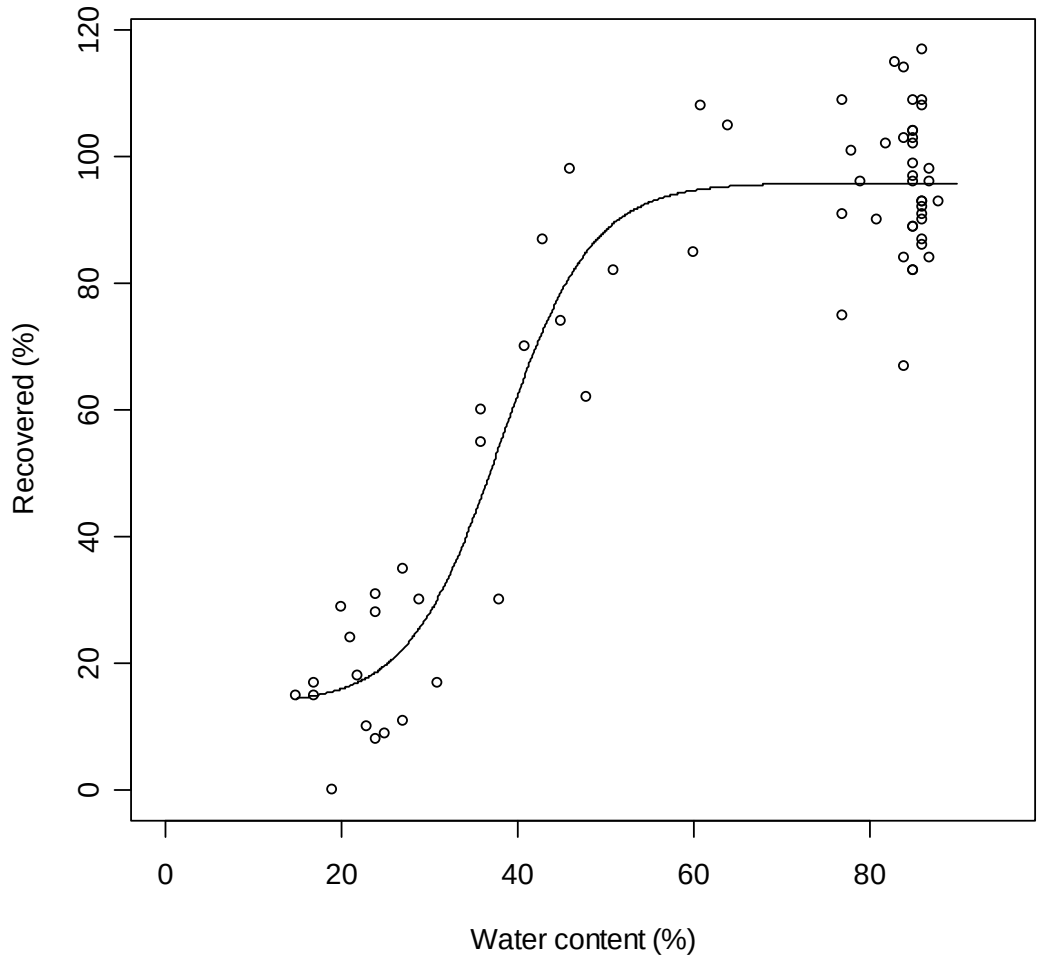


Figure 3-18 Percentage of initial concentration measured against sample water content from the ECT field study (May – July 2006)

3.6 Discussion

3.6.1 Excretion of Ivermectin

This study found similar patterns of ivermectin excretion following subcutaneous injection to several studies in the literature. The peak concentration of 1.3 mg/kg (dw) is within the range of levels (1.1-3.9 mg/kg, dw) measured by other authors (see Section 3.1.1). In this study, peak concentrations were recorded three days after treatment. Published data regarding the day on which peak excretion occurs is more variable. While Lifschitz *et al.,* (2000) reports peak levels after only one day, several other authors report peak excretion to occur 5-6 after treatment. Other authors found peak levels to occur 2-4 days after treatment. As ivermectin levels were not monitored on days 4 and 5 of this study, it is possible that the peak excretion point was missed. While concentrations were monitored up to 18 days after a single treatment, other studies have shown that residues can be excreted at toxic levels up to 34 days after treatment (Kruger and Scholtz, 1995; Kadiri *et al*., 1999). Monitoring for a longer period of time would lead to a more complete understanding of ivermectin excretion profiles; however the current study sufficiently demonstrates that ivermectin excretion is declining slowly after day 8.

The replicate measurements were quite variable. It may have been possible to reduce the variability by including more replicates. However, higher variability may be expected due to the specific circumstances of this study. Many studies in the literature were performed using a small number of cattle (e.g. 4) of the same age or weight, e.g. pharmacokinetic studies using animals from experimental facilities. This approach was not feasible in this study. The farm's non-lactating dairy cattle were all pastured together in the same field. It was not feasible to separate a small group of cattle and collect samples from only that group due to practical considerations, such as the presence of only one water station. An alternative approach was devised, where the manure was collected from the first five cattle to defecate after arrival at the site. Although the results are more variable than those in the literature, this variability may be more indicative of a

realistic scenario where a larger group of different sized cattle pastured together will all be treated.

Two metabolites were demonstrated to be excreted along the with parent compound into the pasture, positively identified as 24-hydroxylmethyl H_2B1_a and 3"-O-Desmethyl H_2B1_a. Unfortunately, due to the difficulty in preparation of standards of these two metabolites it was not possible to definitively quantify the excretion of these two metabolites. However, an indication of the excretion profile was provided by calculating the concentration of two suspected metabolites in terms of ivermectin equivalents. This is supported by the findings of Halley *et al*., (1992) who found 24-hydroxylmethyl H_2B1_a and 3"-O-Desmethyl H_2B1_a to constitute up to 36% and 12% respectively of the applied dose to cattle six days after treatment.

In addition to ivermectin, these metabolites may also have anti-parasitic properties as functional groups (namely the sugar group and a hydroxyl group) key to the activity of the parent compound are still intact (Fisher & Mrozik, 1989; Shoop & Soll, 2002). It is therefore possible that effects studies performed using manure spiked with test compounds may underestimate the toxicity of excreted ivermectin. It may be worth considering performing toxicity studies using manure collected from treated animals, where the concentration of the parent compound and ideally, any major metabolites are quantified, an approach employed by Halley *et al.,* (2005) for testing the toxicity of eprinomectin to earthworms, alongside more traditional tests.

3.6.2 Dissipation and the Effect of Water Content

The results of the persistence field study indicate there was no degradation of ivermectin residues during the lifetime of the dung-pats, which in this study was between 38 and 71 days. The duration of the study was limited by the time it took for the manure to degrade in the field. The degradation half-life of ivermectin in manure therefore exceeded 38 days. This level of persistence has been reported by several other authors following field studies conducted under a range of conditions and locations, e.g. autumn and spring, and Denmark and

Argentina (Sommer & Steffansen, 1993; Iglesias *et al.*, 2006; Suarez *et al.*, 2003). However, several studies have reported significantly faster dissipation under 'summer' conditions, with reported half-lives of less than 7 to 14 days (Lumaret *et al.*, 1993; Halley *et al.*, 1989b).

Our studies, conducted under both controlled laboratory conditions and environmentally relevant field conditions, both demonstrated clear evidence for a strong, positive influence of manure moisture content on the extraction efficiency of ivermectin. There is an apparent water content threshold of 40 – 50%, above which the extraction recovery of ivermectin was good (usually 80 – 110%). Below this threshold, there was a sharp decline in the proportion of initial ivermectin recovered. Although the mechanism for this effect is not known, the physical properties of the organic matrix in manure are likely to change in response to drying, potentially trapping the ivermectin molecule. When water was added to the drier manure samples from the field study (and sufficient time allowed for water to be absorbed) seven times more ivermectin was extracted. Ivermectin residues in dried manure are therefore not non-extractable residues or irreversibly bound, but can be released by soaking for period of time. This indicates that previously 'unavailable' ivermectin residues in dry manure (after a period of dry or warm weather) may be released again following a period of rainfall.

The studies reporting rapid rates of degradation are those conducted during 'summer' conditions, e.g. DT_{50} of 7-14 days in summer and complete degradation after 6 days in Spain (Lumaret *et al.*, 1993; Halley *et al.*, 1989b). Given the strong, demonstrated effect of sample moisture content on ivermectin extraction efficiency (and its reversibility) it seems likely that these studies were not measuring degradation of ivermectin residues but that the decrease in extractable ivermectin was actually an artefact of the sample moisture content. Adding water to drier samples prior to extraction (and allowing sufficient time for the water to be absorbed) may have yielded significantly higher ivermectin levels.

It is possible that residues in drier manure, unavailable for extraction, are also biologically unavailable. This has been demonstrated for other hydrophobic compounds in soils (e.g. Chung & Alexander, 1998; Bowmer, 1991). For example, in a study by Morrison *et al*., (2000) the availability of the insecticides DDT and DDD to earthworms was markedly reduced (by as much as 54%) after 190 days of aging in soil. However, in the case of ivermectin the diminished bioavailability of residues is only temporary, with residues re-released when the matrix is re-hydrated (e.g. following rainfall).

These results reinforce the importance of thorough analytical method validation, in particular, taking account of changes that samples may undergo in response to variable environmental conditions, or sample aging which may affect the analyte extractability. Not only should method validation be undertaken in the appropriate matrix (e.g. cattle dung or soil) but the particular matrix characteristics also need be taken into account, such as diet or animal age in the case of manure, pH, soil type and moisture content. Ideally, methods should be developed and validated in the matrix in which the samples will occur.

In assessing exposure of dung fauna to ivermectin, it is important to know the potential for residue degradation within the period of time the manure is attractive to dung fauna. Dung flies tend to be attracted to only very freshly deposited manure for feeding and oviposition, and dung beetles tend to be attracted to freshly deposited manure for feeding and to older manure for laying eggs (1991; Hirschberger & Degro, 1996), whereas fly and beetle larvae take between 2 to 6 weeks reach pupation (Hirschberger & Degro, 1996). The period of interest is therefore only the period of time before pupation, which will often occur in soil beneath the manure. Evidence from this and several other studies suggest there is no degradation in ivermectin residues within this period of time (Sommer & Steffansen, 1993).

Low levels of ivermectin were detected in the soil directly beneath the pats, confirming the low potential for residues to be transferred to the soil. Mean concentrations of 57 µg/kg ww (Figure 3-14) were highest 71 days into the study

in the top centimetre of soil, once the pats had completely degraded. Erzen *et al.*, reported much lower concentrations of up to 1.0 µg/kg ww detected in the soil from a pasture where sheep were treated with abamectin (Erzen *et al.*, 2005). This is to be expected however, since they also found lower concentrations in dung, and the concentrations found in this study decrease rapidly with soil depth. To our knowledge, this is the first time ivermectin has been monitored in the soil directly beneath manure from treated cattle, under natural, pasture conditions.

These results confirm that although ivermectin has very low potential for transport in the environment, residues may still be transported into the soil. As ivermectin sorbs so strongly to organic matter (Halley *et al.*, 1989a), it is unlikely these residues were transported in the aqueous phase but more likely to be remain bound to the organic matter in the dung pat and incorporated into the soil by the activity of dung and soil fauna.

If we assume soil has an estimated moisture content of 11%, the mean concentrations measured on day 71 in the field study are equivalent to 63.2 µg/kg dw (approximately 56.25 µg/kg ww). At these peak concentrations the analytical methods used show good precision and accuracy (see Tables 2-8 and 2-9), so results are reliable in this range, even though at lower concentrations they are not. This is an order of magnitude lower than the lowest available NOEC for ivermectin in soil (300 µg/kg dw, *Folsomia fimetaria,* Jensen *et al*. 2003), (see Table 1-7).

It therefore appears that exposure to soil fauna is limited. Transfer of ivermectin into the soil is limited and the peak concentrations detected are well below levels known to affect the most sensitive soil fauna species. There is still the potential for ivermectin residues to accumulate in certain areas frequented by cattle due to the persistence of residues in soil. Given the low degradation rates reported in soil e.g. half-lives of between 93 and 240 days (Halley *et al.*, 1989b) the next stage is consider persistence and accumulation of these residues in the environment. However, exposure to soil organisms will be further limited due to

the spatially heterogeneous exposure, since the exposure will only occur at the soil/dung interface.

Subsequent to the initial submission of this thesis, this phenomena has been investigated in soil by Thiele-Bruhn, *et al.*, (Thiele-Bruhn *et al.*, 2010). The authors suggest that the sorptive properties of soil organic matter depend on the structure and rigidity of the matrix. They hypothesise that the rigidity and therefore the sorptive properties of soil organic matter depend on water content and water contact time.

3.6.3 Dissipation in Aquatic Mesocosms

In the aquatic mesocosms the dissipation of ivermectin from the water phase was rapid, with a mean DT_{50} of 4.2 days. However, rates reported for avermectin dissipation in clean water, without sediment or particulate matter were significantly faster, with rates of 4.5 to 12 hours reported. The results from tests performed in the presence of either particulates or sediment are similar to those achieved here. Wislocki *et al.,* reported a DT_{50} of 4 days for abamectin in artificial pond water (Wislocki *et al.*, 1989) while Loffler *et al.,* report a DT_{50} of 2.9 days in a water-sediment test system (Loffler *et al.*, 2005). Ivermectin residues that are strongly sorbed to particulate matter either in the water or in the sediment may not be susceptible to photodegradation.

Modelling the water-sediment system in this study showed ivermectin rapidly dissipated from the water compartment and was sorbed to the sediment. This can be attributed to the high affinity of the avermectins to organic matter; the K_{oc} for ivermectin in soil is between 12,600 and 15,700. Significant sorption to the sediment has also been observed in other studies. Loffler *et al.,* (2005) found 16 – 42% of the applied ivermectin sorbed to the sediment of a laboratory water-sediment system. The sorbed proportion in our semi-field mesocosm study is similar, at approximately 45%.

It has been unclear to what extent degradation in the water, degradation in the sediment and sorption to the sediment or suspended particulate matter drive the

dissipation of residues in aquatic systems. Modelling the water-sediment system demonstrated the dissipation observed in the water phase could not be explained by sorption to the sediment alone. In laboratory tests ivermectin has been shown to rapidly photo-degrade with a half-live of 0.5 days reported (Halley *et al.*, 1993). The additional degradation in water observed in this study could therefore be attributed to photo-degradation.

Ivermectin was, however, highly persistent in the sediment of the mesocosms. There was no discernible decline in sediment residues from either degradation or partitioning back to the water phase for the 275 days of monitoring. In tests with spiked marine sediment and sea water Davies *et al.,* (1998) found the DT_{50} to exceed 100 days. A loss of 30% of the residue was suggested but this was calculated by comparing concentrations determined at the end of the study to the target, or nominal concentrations. As the concentrations measured at time zero were all lower than target concentrations and the recovery of the analytical method was not presented the degradation estimates appear unreliable. Loffler *et al.,* found the DT_{50}, for the overall water and sediment system to be 15 ± 2 days (2005)(2005). The degradation rate in the sediment phase alone was not determined; however, a slow decline was apparent in the graphs, the levels in the sediment appear to have declined by roughly 50% in 90 days.

Considering both the results of this extended investigation and previously published work, ivermectin appears very persistent in the sediment of aquatic systems. It therefore seems likely that if ivermectin does reach surface waters residues will accumulate in the sediment, especially in anaerobic sediments with high carbon content thus posing a risk to sediment organisms.

3.7 Conclusions

This chapter demonstrates how a series of complementary field-based studies can improve our understanding of the fate and behaviour of parasiticides in the pasture environment. These methods are valuable tools in the risk assessment of parasiticides.

The specific hypotheses for this chapter were:

1) The rate of ivermectin excretion from subcutaneously treated cattle in a UK pasture environment can be confirmed.
Excretion profiles measured in this study confirm that ivermectin residues enter the environment at high concentrations as found by other authors and therefore the first hypothesis is correct.

2) Known ivermectin metabolites can be positively identified in excreted manure.
In addition to the parent compound, two metabolites were positively identified in the manure from treated cattle.

3) Extraction efficiency for ivermectin is dependent upon the moisture content of the sample and the effect of moisture content on extraction efficiency can be reversed by re-hydrating the samples.
A laboratory study was designed to confirm the effect of moisture content on extraction efficiency of ivermectin. This study showed that extraction efficiency is strongly influenced by moisture content of dung. This was confirmed, and shown to be a reversible effect through re-analysis of field samples.

4) Ivermectin residues in dung pats do not degrade under field conditions in a UK pasture environment.
After analytical methods were corrected for the effect of moisture content, residues were found to be highly persistent in the environment, with no significant change in concentration observed in the 37 days of the field study. Previous reports of rapid degradation under warmer conditions may be artefacts of the effect of moisture content on ivermectin extraction efficiency.

5) Ivermectin residues are transported into the soil following deposition of dung from treated cattle.

Ivermectin residues can be transported into soil under field conditions. Ivermectin was found in the surface layer of soil under pats from treated cattle, and the concentration increased over time, peaking after the pats themselves had disappeared. Due to the sorptive nature of ivermectin, this transport is unlikely to be the result of leaching; it is more likely to be due to the activity of soil and dung organisms. Concentrations detected in this study were very low and unlikely to pose a risk to UK soil fauna. However, given the marked influence of moisture content on the extraction efficiency in manure, further work should be conducted to see whether this phenomenon also occurs in soil. If a similar effect is present when analysing soil samples, measured concentrations and therefore the risk to pasture fauna, could be significantly underestimated. It may be unwise to extrapolate the results from this study to other regions with different climatic conditions and different soil types (given inherent variability in moisture content of different soil types) until the effect of moisture content on soil extraction has been assessed.

6) The rate of ivermectin dissipation may be assessed under semi-field conditions.

A semi-field study was performed to investigate the fate of ivermectin in water and sediment. It was found that if residues do reach surface waters they will rapidly partition into the sediment where they may persist for long periods of time. This may potentially pose a risk to sediment organisms.

These results indicate a potentially high risk to dung fauna from ivermectin use. Further investigation is required to assess the impact of ivermectin use on dung fauna, given the findings from this chapter regarding the extent and variability of ivermectin concentrations in dung pats which are likely to occur under field conditions. This will be addressed in the next chapter.

4 Modelling the Impact of Parasiticides on Pasture Non-target Organisms

4.1 Introduction

Risk assessments are performed on new and existing chemicals to ascertain if they will adversely affect the environment. The detrimental effect on non-target organisms may be considered at different levels, from the individual organism to the ecosystem to which they belong, depending on which (biological) level we are interested in protecting. While most of the emphasis in ecotoxicology is on assessing and defining concentrations at which toxicants will cause an effect at the individual level e.g. by determining the LOEC or NOEC (lowest observed or no observed effect concentration) for single organisms, it is generally accepted that to assess the ecological relevance or significance of these endpoints requires an understanding of how these endpoints relate to population, community and ecosystem responses (Kuhn *et al.*, 2001; Beketov *et al.*, 2008; Forbes *et al.*, 2008; Forbes *et al.*, 2008). For example it may be helpful to know: if a population will be damaged in the long-term; if the affected population is likely to recover from the impact; if there is a significantly increased risk of extinction; or if there are indirect effects on other non-target organisms, e.g. a knock-on effect on predators. For compounds such as pesticides, human or veterinary pharmaceuticals it may be unavoidable for a certain number of individuals to be lost. Some mortality of non-target organisms may be acceptable if the population of those organisms can be shown to be unaffected in the long-term; for example, if it can be demonstrated that the population will recover within an acceptable amount of time. Exposure to these compounds needs to be considered in more detail, and the effects of that exposure on the population or community in the longer term assessed in order to produce a more complete assessment of the risk which the chemical presents in use (Floate *et al.*, 2005; Sherratt *et al.*, 1998).

For the environmental risk assessment of new parasiticides guidelines have been developed by VICH (VICH, 2004), and more recently a technical guidance

document has been developed by EMEA (CVMP, 2008) to complement the VICH Guidelines. Due to the special concern for dung fauna, when parasiticides are to be used for pastured animals, the VICH guidelines dictate that Phase II tests are necessary, whether or not this would have been triggered by the results of Phase I. The initial, conservative risk characterisation process for assessing the risk to dung fauna, and the next options to further refine the risk assessment have been outlined in Chapter 1.

If an unacceptable risk to dung fauna is determined following the tiered risk characterisation process detailed in the VICH guidelines and the EMEA guidance document (VICH, 2004; CVMP, 2008), using more realistic (but still conservative) values for the predicted environmental concentration (PEC) then the risk assessment will need to be further refined. For example, for ivermectin, the conservative risk assessment presented in Equation 1-6 can be recalculated as shown in Equation 4-1 using the results of dung fly testing and the excretion data from Chapter 3. As the resulting RQ is higher than one, an unacceptable risk has been found.

$$RQ = \frac{PEC}{PNEC} = \frac{180\ \mu g/kg\ \ fw}{0.93\ \mu g/kg\ \ fw} = 194 \qquad \text{Equation 4-1}$$

Where PEC is the predicted environmental concentration and the PNEC is the predicted no effect concentration. The PEC of 180 µg/kg is the peak measured concentration in fresh weight (Chapter 3) and the PNEC is derived by dividing the NOEC of 9.3 µg/kg fw (Rombke *et al.*, 2009) by an assessment factor of 10 (VICH, 2004).

When parasiticides reach this stage in the risk assessment process, there is no longer any formal advice for further refinement in the guidelines or guidance document. Instead, the applicant who is seeking a marketing authorisation for the parasiticide is directed to contact regulatory authorities for further advice. There are however a number of possibilities for refinement of a parasiticide risk assessment, from testing under field conditions to derive a more realistic PNEC,

enabling the use of a lower assessment factor, or methods such as field monitoring and population modelling to assess the population level response.

4.1.1 Field-based toxicity studies

One approach that may be used to refine the risk assessment is to conduct a dose-response type experiment under field conditions. One such method is described by Lumaret *et al.,* (1993), this approach has subsequently been used by Inglesias *et al.,* (2006) as well as in the ERAPharm project in a field study in York. In these studies, cattle were divided into groups and treated with the same dose of parasiticide at different times. This enabled manure samples containing a range of concentrations to be collected on one day. The manure from each group was then homogenised and a series of artificial pats were placed in the field. At each sampling time point pats of each concentration group were removed and the invertebrates present identified and counted. In the ERAPHARM study, in addition, samples of the soil directly beneath the pats were taken, to monitor the presence and abundance of soil arthropods, pupating dung fauna and nematodes. The fate of the parasiticide may be simultaneously monitored, using methods described in Chapter 3.

Although these studies may, potentially, produce a NOEC for the dung fauna community, they do have a key limitation, i.e. if the parasiticide of interest affects the insects' development (e.g. is toxic to larvae) then counts of larvae, pupae and foraging adults, as opposed to emerging adults, may underestimate toxicity. Indeed, studies noting the abundance of adult coleoptera are more likely to be exhibiting the repellent (or attraction) effect of parasiticide treatment, rather than direct toxicity (e.g. Suarez *et al.*, 2003).

The studies performed by Floate *et al.,* (1998; 2002) and Iwasa *et al.* (2005) offer a more useful approach. In these studies, artificial pats of different concentrations are placed in the field for 5 – 8 days to be naturally colonised by dung insects. The pats are then removed and transported to the laboratory where the resulting insect emergence is monitored. Using this method, a community

NOEC may be determined incorporating the effects of the parasiticides to all developmental stages.

However, these methods still do not consider the long-term, population-level impact resulting from the effect that parasiticide-induced mortality has upon future insect generations. The response of an insect population to parasiticide use is not discernable from dose-response ecotoxicity tests alone. It will depend on the temporal pattern of exposure - determined by parasiticide excretion rates and livestock treatment regimes, the reproductive capacity of the population, the availability of sources of immigration such as areas of untreated cattle, the magnitude of other population stressors such as drought and the capacity of the population to compensate. Exposure to parasiticides is not constant and uniform as assumed by the highly conservative PEC/PNEC calculation used in lower tier risk assessments. There is a more pulsed exposure with manure containing toxic residues occurring for finite periods of time immediately after livestock treatment (if treated on the pasture), following which residues will decline. There is also potential for dissipation in the environment. Toxicity tests based on spiked concentrations may overestimate the effects of rapidly degrading parasiticides, although consideration of dissipation may be of more relevance when considering the impact on the soil compartment. As a result, exposure to parasiticide residues is patchy; non-target organisms are potentially exposed to a mixture of parent compound, metabolites (excreted by livestock) and degradation products (from degradation in the environment) and manure containing a range of concentrations.

At the population scale, aspects of insect ecology become important. Several studies have demonstrated the range of seasonal activity periods occupied by dung insects (e.g. Floate & Gill, 1998; Lee & Wall, 2006a; Gittings & Giller, 1999; Giller & Doube, 1994). The overlap in time (or co-incidence) between the insect of interest and toxic residues has been largely ignored, with the exception of the modelling studies conducted by Sherratt (1998) and Boxall *et al*., (2007). In addition, insects of different life-history characteristics may respond very differently at the population level to the same level of toxicity. A population of a

fast-breeding (multivoltine) fly species may be more likely to recover quickly from exposure to toxic residues than a univoltine beetle.

4.2 Field Monitoring Studies

Field monitoring studies may incorporate these issues, and the response of non-target dung fauna populations to parasiticides have been investigated at the field scale in a limited number of monitoring studies. Abundance of *Scatophaga stercoraria* was monitored in ivermectin- and doramectin-treated grazed pastures in south-west Scotland by Webb *et al.*, (2007) using dung-baited pitfall traps to capture adult flies between April and July in 2002 and 2003. In addition, the non-lethal end-point of wing asymmetry (a developmental abnormality) was examined in pastures containing cattle treated with doramectin. While *Scatophaga* abundance was found to vary with year and season, no effect of avermectin use was detected. Incidence of wing asymmetry however, was found to be significantly influenced by doramectin treatment, when compared to pastured grazed by untreated cattle.

This study raised several important issues when considering field monitoring studies. When designing the wing asymmetry study, care was taken to sample from pastures surrounded by fields also used by avermectin treated cattle, increasing the chances of sampling flies that had actually emerged from avermectin contaminated manure and not from nearby pastures of untreated cattle. Webb remarked that a lack of visible response in insect abundance did not necessarily mean a lack of effect in the population. If the flies travelled far enough and distances to pastures of untreated cattle were close enough then a local decline in abundance may be masked by an influx of adults from the 'untreated' pastures (Webb *et al.*, 2007). Special attention needs to be paid, when considering the spatial scale of monitoring studies, to distances to pastures of untreated cattle which may act as reservoirs of healthy flies. It has been recommended that field investigations into the impact of parasiticide use are conducted on a much larger scale (Wardhaugh, 2005). However, increasing the area under investigation beyond the farm-scale will be difficult to implement due

to increased costs and the availability of cooperative neighbouring farms (Wardhaugh, 2005; Floate *et al.*, 2005).

The study by Webb *et al*., (2007) involved a fast-breeding species. However, population level effects on a slower-breeding insects such as the largely univoltine aphodius species (Gittings & Giller, 1997), may not be apparent for many months or longer and the species may be very slow to recover. Subtle changes to reproductive rates due to exposure to toxic residues may take generations to be measureable (Snell & Serra, 2000). Indeed, slower-breeding species may be particularly vulnerable to adverse changes in their environment due to their lower reproduction potential (Barnthouse *et al.*, 1990). Field monitoring studies sensitive enough to detect effects at the population level of many beetle species would therefore need to operate over a very long timescale, perhaps maintaining the same parasiticide treatment for several years.

When Hutton and Giller investigated the effect of different farm management practices on Irish dung beetle communities, each farm site had been under the same farming practice for at least 3 years (2003). The study ran from May to October and found significantly greater beetle biomass, diversity and species richness on organic farms where no parasiticides, synthetic fertilisers or pesticides were used, compared to intensive and rough grazing farms where parasiticides, synthetic pesticides and fertilizers had been used.

Another issue with field studies is the problem of reproducibility, as the contribution made by other population stressors such as weather and the presence of predators is difficult or impossible to control. An effect caused by a toxicant may be observed only when the population is under multiple stressors which in field studies is again difficult to control or predict. For example, a two year study into the effects of ivermectin on the structure of the dung fauna community in South Africa detected a reduction in species diversity and an increase in the dominance of certain species in a drought year but could discern no effect the following (wetter) year, when the additional population stressor was absent (Kruger & Scholtz, 1998a; Kruger & Scholtz, 1998b).

4.2.1 Population modelling

At later stages in the environmental risk assessment process, a wealth of information is available including: the excretion data which will have formed part of the ADME (Adsorption, distribution, metabolism and excretion) package of data and the results of the laboratory dose-response data (which is obligatory). Moreover there is a considerable amount of information on dung fauna ecology available in the literature. All of these data can be incorporated into population models to estimate impacts on populations of insects. Population models offer a number of advantages including: they are quick to run; they do not entail the cost of detailed field-based investigations; they can be run for a number of years and hence help to identify effects that could occur in the future; and they allow the effects of animal husbandry and parasiticide treatment practices to be explored thus assisting in the identification of risk mitigation strategies. Depending on the results of the modelling, field studies may still be necessary. In those cases, the population modelling may be used to help inform the field study design, perhaps by guiding the investigation to the insects deemed to be the most sensitive at the population level which may not necessarily be those most sensitive to parasiticide residues as population-level response will be influenced by the ecology of the insect.

These models can be age- or stage-structured models, such as the Leslie matrix model (Leslie, 1945); individual based models or meta-population models; and may be spatially explicit, deterministic or probabilistic. Population modelling has been employed to investigate the effect of parasiticides on dung fauna, ranging from simple screening models, to highly complex and spatially explicit models. While these models have not been developed with the assessment of new parasiticides in mind, they do introduce several useful methods. A brief summary of the previously published modelling approaches is presented in Table 4-1.

Table 4-1 Modelling methods previously used to assess impact of parasiticides on dung fauna (after Cooper *et al.*, 2003)

Reference	Type of model	Dung biota	Age or stage structured model	Movement between pats	Parasiticide/s	Life stage used for toxic effect	Livestock & form of administration	Spatial scale	Maximum temporal scale	Model limitations
Boxall *et al.*, (2007) and Cooper *et al.*, (2003)	Simple screening level index	Horn fly, house fly, stable fly	No	No	Doramectin, eprinomectin, ivermectin, moxidectin	Larvae	Cattle, any from survey	Farm	1 year	Very simplistic No consideration of effect on future generations
Sherratt *et al.*, (1998)	Detailed simulation	Beetles and flies	No	No	Avermectins	Larvae	Cattle, subcutaneous injection	Farm	250 days	No consideration of effect on future generations Considers toxicity to larval stage only
Wardhaugh *et al.*, (1998)	Detailed simulation, matrix population model	Beetles, based on *O alexis* & *O binodis*	Yes	Yes	Deltamethrin	Adults, larvae	Cattle, pour-on	Farm	168 days	Compensatory effect of density dependence is ignored Results based on bioassays Limited model duration constrains assessment of effects to slower breeding insects
Wardhaugh *et al.*, (2001a)	Detailed simulation, matrix population model	Univoltine and multivoltine (slow and fast breeding) beetles	Yes	Yes	Ivermectin	Larvae	Sheep, controlled release & oral	Farm	200 days, one flight season	
Vale and Grant (2002)	Detailed simulation, including density dependence, insect dispersal, seasonal abundance	3 hypothetical species; fast, medium and slow breeders	Yes	Yes	Synthetic pyrethroids	Adults, larvae	Cattle, continuous treatment at intervals of 10,20, 40 days	Landscape scale, 1-9 km^2 treated areas in different arrangements, surrounded by untreated areas	2000 days (~5.5 years)	Highly complex with large number of assumptions

The main advantage of simpler models, such as the one developed by Boxall *et al.,* (2007) is that they require little data and use data that would be readily available for the testing of new compounds along with insect activity periods (e.g. flight periods). Boxall's model uses toxicity data generated for the organism in question (such as would be provided by the new OECD dung fauna testing guidelines) and the withholding periods to identify the maximum period of time residues will occur in the field. Information on insect seasonality is used to predict the proportion of time that the life-stages which are sensitive to residue toxicity will be exposed. This simple, screening level model does not consider the long-term effects, such as the effect removing a proportion of the population may have on future generations or the combined effects that toxicity-induced mortality to several different life-stages may have on abundance.

The models developed by Sherratt *et al.,* (1998) were the first attempt to quantitatively estimate the degree of exposure from the co-incidence of toxic residues and the seasonal activity of the insects. Analytical models were employed to determine the number and proportion of individuals killed as a direct result of avermectin use, for a number of insects over several treatment scenarios. Again, these models do not consider the possible implications in terms of future reproductive performance or the possible compensatory effects of density-dependent processes. The authors remarked that the complexity of density-dependent and density-independent processes make prediction of long-term dynamics with any accuracy very difficult. In addition, as these models were developed for avermectin use, they do not consider the combined effects when residues are toxic to more than one life-stage, such as synthetic pyrethroids which are toxic to adults and larvae of several dung beetles.

Wardhaugh *et al.* (2001a) used an age-structured matrix model to explore the potential effects of parasiticides on dung beetle populations. The model compared the effect of single and multiple doses, different application methods and the effect of treatment timing with respect to different times after insect emergence. The modelled insects were based on composites of several insects with similar life-histories. With sufficient ecological understanding this

approach could be extended to encompass a range of theoretical insect species, such as fast-, and medium-breeding flies and fast-, medium- and slow-breeding beetles. The models developed by Wardhaugh *et al*. (1998; 2001a) included toxicity modifiers to both fecundity and to age-specific survival terms and so could consider toxic effects to different life-stages such as synthetic pyrethroid toxicity to larvae and adults. In addition, the matrix population method allows for the inclusion of non-lethal effects such as changes to development rates. However, the authors found further experimental data was required to include effects on fecundity and immature development. The model output was presented in terms of a relative activity index, calculated by dividing the cumulative total of adult beetles in the treated scenarios by the cumulative total in the control scenarios for each point in time and displayed in graphs.

One of the studies compared the population response of slow- and fast-breeding dung beetles, with egg to reproductive adult development times of approximately 90 and 50 days respectively, to a slow-release formulation of ivermectin (Wardhaugh *et al.*, 2001a). The authors explain that the fast-breeding population was more severely effected because toxic residues were released for up to 100 days. This may be understandable as the duration of exposure is at least the duration of two generations. However, the lag period between the start of exposure and visible effect will be longer for the slower breeding beetles and as the model ran for only 100 days after the start of exposure, the full extent of the effect was unlikely to have completed its course. These models do not incorporate density-dependent processes and so are unable to incorporate the potential for the population to recover from parasiticide exposure. In addition, these models are based on bioassay results (using manure collected from treated animals) rather than dose-response toxicity testing and excretion profiles.

Vale and Grant (2002) presented a very detailed, age-structured simulation exploring the effects of continuous treatment on non-target dung fauna using three hypothetical insect species: fast-, medium- and slow-breeders with both adult and pre-adult insects influenced by toxic residues. This complex model demonstrates the relative impact of a large number of factors, such as: the

attractive life-span of pats, frequency of pat occupation, variation of pat toxicity within treatment interval (e.g. a non-linear excretion profile), changes in dispersal rates (due to age, population density, density-dependence of recruitment and death), and the size and shape of the treated area (Vale & Grant, 2002). The model was aimed at investigating the impact of continuous pyrethroid treatment at intervals of 10, 20 and 40 days for the control of tsetse flies, a treatment scenario very different to those employed in the UK where cattle tend to be housed in the winter months and treated on turn-out. Although this scenario is not relevant to ivermectin use in the UK, it is a good illustration of how such models could be used to assist risk assessments.

None of these models included sub-lethal effects. However, the age- and stage-structured approaches employed by Wardhaugh *et al.,* (1998; 2001a) and Vale and Grant (2002) could include parasiticide-induced reduction in fecundity and increased development times if the relevant data were available. The use of theoretical species in these models is a useful approach for dealing with a lack of life-history data to feed into the models. The models demonstrate several aspects that would be useful in the assessment of parasiticides. However, as each was developed with a different question in mind, none are entirely appropriate for the assessment of a new parasiticide.

4.2.2 Selection of Modelling Approach

The aim of the study was to develop a model that could be used to investigate the impact of parasiticide use on a range of different dung fauna species: from flies, in which commonly only the larvae are exposed because the adults are not dung dependent (e.g. *Scatophaga*); to beetles, which may be exposed through diet as adults as well as larvae. The model needed to be able to accommodate toxicity induced changes to different life-cycle parameters such as: reductions in fecundity, development time, larvae survival, emergence as adults and adult survival. Where relevant, these effects can be measured in the new dung fly toxicity tests, OECD no. 228, and the beetle tests, for which guidance is currently under development by the DOTTS (Dung Organism Toxicity Testing) group.

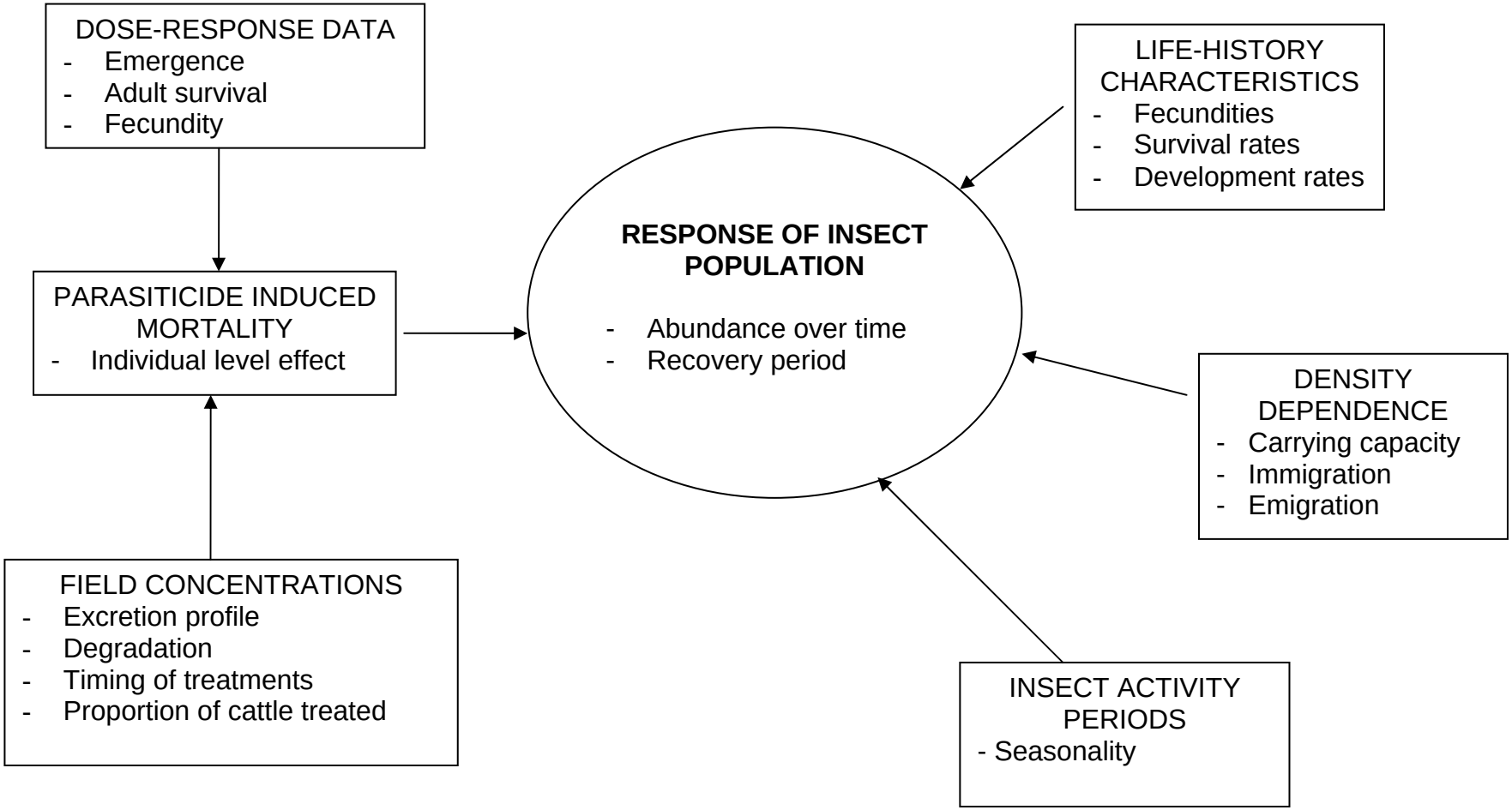


Figure 4-1 Conceptual model for parasiticide effect on non-target dung fauna at the population level

Figure 4-1 illustrates the conceptual model, summarising the parameters to be employed.

A stage-structured matrix modelling approach was selected as a pragmatic approach based upon the information available in the literature. Previous age-structured matrix models showed the approach had merit when used for modelling populations of dung fauna, but the previous work was not designed with risk assessment in mind.

Stage-structured models are relatively straightforward to set-up with software such as Matlab and are an established ecological modelling method (2001). The model was developed and assessed for one species but its potential for extension to a suite of theoretical species was also considered. The model was built to easily accommodate three groups of information: chemical fate, toxicity and ecology. A rationale for selection of the model parameters is given in the following sections.

The response of a population may be assessed in terms of a number of endpoints, including: population size, population growth rate (r), equilibrium (steady state) abundance, probability of (local) extinction, time to extinction, minimum viable time for population to recover and the probability of recovery (Akçakaya & Stark, 2008; Menzie *et al.*, 2008).

In this study, the main endpoint used is population abundance and, to a lesser extent, time to recover. Abundance is a useful endpoint as it effects the functioning of the ecosystem, reduced abundance increases the risk of local extinctions following environmental and demographic variation and because society places value on abundance (Menzie *et al.*, 2008).

4.2.3 Aims and Objectives

To aid in the risk assessment of a new parasiticide, a model is required which can use the information which is already available at this stage of the registration process: these data are 1) the measured excretion rates produced as part of the

absorption, distribution, metabolism and excretion (ADME) investigation; 2) the results of any fate assessments; 3) insect seasonality to determine a realistic exposure; 4) the results of the dose-response tests to dung insects and 5) insect life-history characteristics either from the existing information in the literature or the development of theoretical species or insect scenarios.

The aim of the work described in this chapter was to develop a modelling strategy to assess the impact of parasiticides on non-target pasture fauna. The model was designed to use the data that would be available as part of the package of information for new compounds. Ivermectin was used as a case study compound to demonstrate the utility of the model.

The underlying assumptions to the work were:

1) The impact of parasiticide use on dung fauna populations is dependent on the inherent toxicity of the compound, the degree of exposure, and the life-cycle characteristics of the species of interest; and
2) The impacts at the population scale can be modelled based on data that companies will have generated during the environmental risk assessment process for a parasiticide.

This leads to the following hypothesis:

- A simple Leslie matrix model can be used with existing information in the literature and the data derived from field studies (Chapter 3) to estimate or identify potential risks to dung fauna.

4.2.4 Ecological Aspects

Different regions are characterised by different dung fauna communities. In the lower latitudes, *Scarabaeidae* tends to dominate the communities, while in more temperate regions a more diverse community of dung beetles and flies may exist (Hanski, 1991a). Moving towards warmer and drier areas in the southern temperate zones, *Aphodius* tends to give way to *Scarabaeidae* (Hanski, 1991a).

When the same insects are present they may well still have different seasonality. For example in the Mediterranean, *Aphodius constans* has a reversed seasonality to cope with the warmer and drier weather, ovipositing from December to March (Lumaret. & Kirk, 1991).

Previous work (e.g. Wardhaugh & Rodriguezmenendez, 1988; Vale & Grant, 2002) has shown that the co-occurrence of insects (governed by their seasonality) and livestock treatments can make a large difference to the impact of veterinary medicines on non-target dung fauna. One useful approach may be to develop insect scenarios for different regions using the husbandry methods likely to be used in those countries and the regional seasonalities of those species. It was decided therefore to focus the current modelling on north temperate species. The seasonality or phenology of north temperate dung fauna species have been investigated in detail for some species and in more general monitoring studies and is summarised in Table 4-2. Gibbons (1987) and Parker (1970) published detailed information on adult *Scatophaga* activity periods which tend to be active April/May to mid November with a characteristic dip in abundance measured in the hottest months. A detailed account of the seasonal abundance and life-history characteristics of the dung beetle *Aphodius rufipes* is given by Holter (1979). Gittings and Giller (1997) reported the results of a three year study monitoring the seasonality and reproductive strategy of 13 Aphodius species in two sites in County Cork, Ireland. The authors used laboratory studies to investigate the beetles' life-history parameters, including their egg development strategy and oviposition site; and used baited pitfall traps to investigate their successional occurrence in the pat (preference in pat age) and adult flight period. Lee and Wall (2006a) investigated seasonal abundance and successional occurrence in a two year study conducted near Bristol in the UK where artificial pats were placed in the field for seven days to be naturally colonised before transportation back to the lab to monitor emergence. The adult flight periods of both *Diptera* and *Coleoptera* were reported. In general, *Diptera* appeared significantly later in the season than *Coleoptera*, with the most abundant dipterans showing distinct peaks in abundance, while most of the beetles tended to occur at relatively low levels of abundance over longer periods.

Stage- or age-structured models require a considerable amount of information on the life-history characteristics of the organism, such as: fecundity, longevity and survival rates at different life-stages. Table 4-2 shows a summary of the life-history characteristics of some dung fauna species of north temperate zones. For some organisms, such as *Scatophaga stercoraria* an insect used to demonstrate many key biological concepts, the life-history characteristics are well understood. However, for many dung fauna insects the key life-history information is scarce.

The new OECD dung fly testing guidelines recommended the use of *S. stercoraria* as one of the flies on which to test new parasiticides. For this reason, and because more data was available on the life-history characteristics of this dung insect, *S. stercoraria* was selected as the initial test organism. However, the *Scatophaga* model was developed with the aim of being easily adapted for other dung fauna species, either using species-specific data or for theoretical species where life-history characteristics from several similar organisms are used, such as the approach used by Wardhaugh et al., (1998; 2001a).

Simulating population dynamics without the inclusion of some form of density dependence assumes an unlimited resource (i.e. manure) supply. This is clearly unrealistic as there is a finite amount of manure available in the pasture, controlled by the number and age of the cattle in the pasture. In a pat occupied by developing larvae at high densities, the risk of the manure being used up before an individual larva completes its development is higher. If the manure runs out before the insect has completed its development then it will die and perhaps only the fastest developing insects will survive.

Table 4-2 Life-history characteristics of some North temperate dung fauna species

Insect and seasonality	Adults			Eggs			Duration of larval stage	Duration of pupal stage	Overall Pre-adult
	Pre-oviposition period	**Adult survival (lifetime)**	**Lifetime fecundity**	**No. eggs per clutch (clutch size) or brood balls**	**Frequency of clutches or brood balls**	**Duration of egg stage (time to hatch)**			**Duration of egg to newly emerged adult**
Scatophaga stercoraria (yellow dung fly) Spring and autumn peaks (Gibbons, 1987; Strong & James, 1993)	2 weeks with excess food (Blanckenhorn *et al.*, 2001) 13.3d at 19-20°C (8-21d range) (Gibbons, 1980) Females ~ 3 weeks, males ~ 4-5 days to (Parker, 1970)	Adult survivorship curves (with increasing egg batches) (Gibbons, 1987) Mean longevity 71.0 days (± 25.9 SD) (Jann & Ward, 1999) Mean longevity (±SD) after copulation: 32.00 ± 4.05 days (n=16) (Martin *et al.*, 2004)	Females can complete at least 7 gonotrophic cycles (Gibbons, 1987)	~40 (Martin *et al.*, 2004) With high food ~ 60 eggs/ clutch, smaller clutch size with less food (Jann & Ward, 1999) No. eggs laid is correlated with food eaten (0.96) (Gibbons, 1980)	6-7d to mature another clutch of eggs (Gibbons, 1987) Smaller females produced 2 more clutches (Jann & Ward, 1999)	Within 24h at 20°C Blanckenhorn, 1997 Eggs hatch after 1-2 days (Hirschberger & Degro, 1996) Mean proportion of eggs to emerge: 0.82 (± 0.08 SD) DOTTS	~ 2 weeks (Hirschberger & Degro, 1996)	Inferred: if total ~28d, then ~13d for pupae	24- 42 days in SW England (Strong & James, 1992) 3-4 weeks (Parker, 1970) Temperature dependent, at least 4 weeks in spring (Blanckenhorn *et al.*, 2001), 21-23d at 20C, 35d at 15C, (Blanckenhorn *et al.*, 2001)
Neomyia cornicina Peaks in late August and early September (Wall *et al.*, 2008)				Gravid female matured a mean 28.8 eggs (Wall *et al.*, 2008)					
Neomyia viridescens Peaks in late August and early September (Wall *et al.*, 2008)				Gravid female matured a mean 37.1 eggs (Wall *et al.*, 2008)					
Aphodius ater April to June (Hirschberger, 1999; Gittings & Giller, 1997)	Eggs laid 4-8 days after feeding following overwintering (Hirschberger, 1999; Gittings & Giller, 1997)			Laid singly in crust (Gittings & Giller, 1997)			April to July 35-45 days (Gittings & Giller, 1997) 4-6 weeks (Hirschberger & Degro, 1996)		
Aphodius constans Mainly late winter and spring in UK (1991) Active in winter with summer diapause in Mediterranean (Hempel *et al.*, 2006)							~ 4 weeks in (Hempel *et al.*, 2006)		~ 9 weeks (Hempel *et al.*, 2006)

Table 4-2 continued - Life-history characteristics of some North temperate dung fauna species

Insect and seasonality	Adults			Eggs			Duration of larval stage	Duration of pupal stage	Overall Pre-adult
	Pre-oviposition period	Adult survival (lifetime)	Lifetime fecundity	No. eggs per clutch (clutch size) or brood balls	Frequency of clutches or brood balls	Duration of egg stage (time to hatch)			Duration of egg to newly emerged adult
Aphodius depressus mid May to early Sept (Gittings & Giller, 1997)				4-18 in soil, simultaneous egg development (Gittings & Giller, 1997)			Dung, fast (20-25 d at 19-20°C) (Gittings & Giller, 1999)		
Aphodius erraticus Mid May to early August (Gittings & Giller, 1997)				Laid singly next to brood masses in soil Sequential (Gittings & Giller, 1997)			20-25 days (Gittings & Giller, 1997)		
Aphodius fimetarius Early April to mid October (Gittings & Giller, 1997)				Singly in crust (7+7 Sequential)					
Aphodius. fossor May to mid July (Gittings & Giller, 1997)				Laid singly in crust, sequential			30-35 days (Gittings & Giller, 1997)		
Aphodius rufipes Mid May to end of August (Gittings & Giller, 1997)				4-14, simultaneous (Gittings & Giller, 1997; Holter, 1979) Oviposition is density dependent (Holter, 1979)		~ 5 days (Hirschberger, 1998; Holter, 1979)	30-35 days (Gittings & Giller, 1997) 5-7 weeks (Holter, 1979)		
Aphodius rufus Early July to late September				Laid singly, sequential (Gittings & Giller, 1997)			Dung, 35-45 days (Gittings & Giller, 1997)		

There is clear evidence for density-dependent fecundity rates in dung fauna. In field and laboratory studies Holter (1979) demonstrated that the number of eggs laid by *Aphodius rufipes* within a pat was density-dependent. This variable rate of oviposition could prevent over-exploitation of manure (insects losing their food and shelter before completion of growth) and appeared to maintain a relatively constant initial larval abundance of 100-150 individuals per pat (Holter, 1979). Hirschberger (1996) examined the oviposition behaviour of *Aphodius ater* in response to *Scatophaga* larvae abundance in a series of choice experiments. The authors surmised that beetles were able to avoid laying eggs into manure likely to have been depleted by fly larvae before they had completed their own development by avoiding laying eggs in manure containing high densities of fly larvae and laying eggs into older manure (Hirschberger & Degro, 1996). Laboratory studies by Amano (1983) and Sigurjonsdottir (1984) have demonstrated the impact of population density within a pat on the probability of emergence as adults. Both these studies show a clear decline in the proportion of introduced insects successfully emerging as adults with an increase in density.

Both experimental and theoretical studies have shown that density dependent processes can effect a toxicant's impact on population levels (Moe, 2008). If density dependence is included in the model, constraining the upper limits of the population density in the 'control' (no parasiticide) population then there is potential for the population exposed to toxic residues to recover — for insect abundance to reach that of the 'control' population.

4.2.5 Exposure Aspects

The organism's exposure will be directly influenced by the chemical fate of the compound. Firstly, the concentration of the compound in freshly excreted manure will change over time after treatment, depending on the pharmacokinetics of the compound and the application method or formulation used. The rate of excretion from the animal is part of the information generated in the ADME tests.

In addition, there may also be degradation of the active compound in the manure. If residues degrade within the period the manure is attractive to organisms, then this will effect the concentrations the organisms are exposed to. For example, the adult beetles *A. fimetarius* and *A. fossor* arrive late in the pat succession (Gittings & Giller, 1997) and if exposed to a more rapidly degrading parasiticide then they will not be exposed to the same concentrations as those insects attracted only to freshly excreted manure (e.g. *Scatophaga*). Lastly, concentrations reaching the field over time will obviously depend on the number and timing of treatments and proportion of cattle treated.

4.3 Case-Study: Ivermectin

Application of the matrix model was demonstrated using ivermectin as a case-study compound using the excretion profile reported in Chapter 3 and the treatment strategy recommended by Merial that calves are treated with Ivomec Classic Injection 3, 8 and 13 weeks after turn-out (NOAH, 2008). In the case of ivermectin, there is no discernable degradation for up to 39 days (see Chapter 3), and so concentrations in manure are considered constant. The new OECD fly tests were ring tested (an inter-laboratory validation of the method) using ivermectin (Rombke *et al.*, 2009). The raw results from one of the tests contributing to the ring testing using *Scatophaga stercoraria* were kindly provided by Dr Jorge Römbke of ECT.

4.4 Methods

4.5 Model Development

Two models were developed using Matlab 7.1 / R14 (Mathworks Inc., 2005) with parameter data derived from the literature and the exposure concentrations from Chapter 3. One model ignored the effect density dependence has on emergence rates, and one included it. The key assumptions were based on data derived under field conditions where possible and appropriate. Modelling was

confined to females and, for ease of model development, the age class intervals were set to one day.

The model was based on the Leslie matrix (Leslie, 1945) method where the age-structured population changes according to the equation:

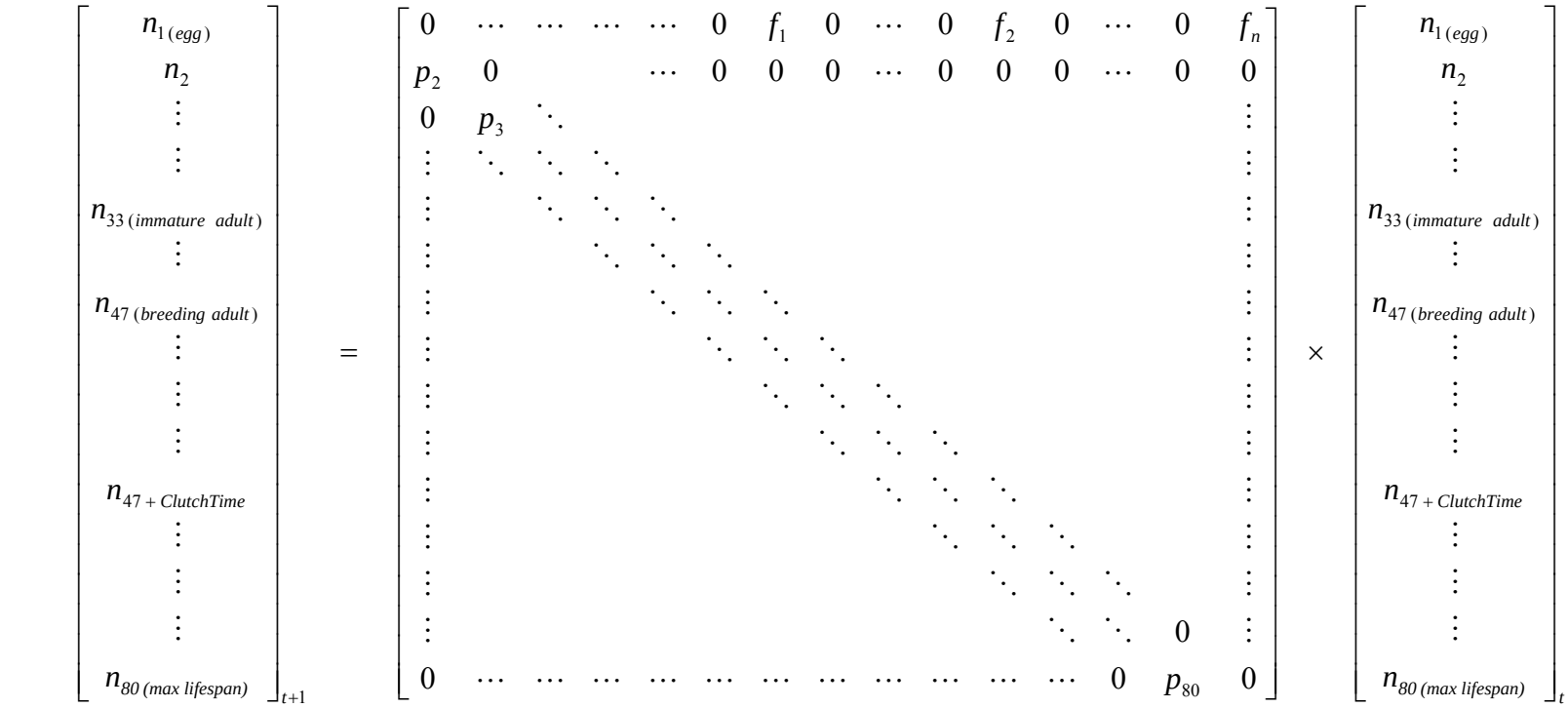
$$N_{t+1} = M.\, N_t \qquad \text{Equation 4-2}$$

where N_t and N_{t+1} are the vectors of n age classes (with daily intervals) at times t and t_{+1} respectively, and M is the n x n matrix of age-specific fecundity and survivorship terms (Wardhaugh *et al.*, 1998). The model can also be written out in an element-wise form as in Equation 4-3.

The Leslie matrix consists of a number of columns and a number of rows equal to the maximum number of days in the fly's life (MaxLifeSpan), from egg to death. Each column in the matrix describes the transition from one age-stage to the others. The first column describes what will happen to the first age group, n_1 (eggs 1 day old), with subsequent columns corresponding to later age-stages, through to $n_{MaxLifeSpan}$. The Leslie matrix (M) is composed of zeros except for the elements describing the probability of moving from one age-stage to the next (p_2 to p_{80} in Equation 4-3) and the fecundity terms on the first row (f_1 to f_n in Equation 4-3). These terms describe the number of female eggs (ClutchSize) that flies of particular ages will lay the next day.

The gaps between the fecundity terms correspond to the number of days required for a fly to mature a new clutch of eggs (ClutchTime). The fecundity terms start from $n_{EggTime+ImmatureTime}$ at intervals of ClutchTime until $n_{MaxLifeSpan}$.

The initial model output is called the PopulationMatrix, a series of vectors representing the size of the population in each age group for a given day [N_1 N_2 N_3 … $N_{Duration}$] concatenated into a single matrix. These matrices are provided at the end of each model run for both control and treated populations.



Tomorrow's population vector *Leslie Matrix* *Today's population vector*

Equation 4-3

4.5.1 Insect Parameterisation

The following section describes the life-history information used to develop the model to simulate the population dynamics over a season in a pasture of untreated cattle. These parameters can be adjusted within the matrix during a simulation depending on the current exposure, according to results obtained from toxicity tests. The model is developed so that these parameters may be easily changed to model the effect of parasiticides on other dung fauna species.

Development Times

Scatophaga development rates are temperature dependent (Blanckenhorn *et al.*, 2001). Under field conditions in south-west England, Strong and Wall (1992) reported egg to newly-emerged adult development rates of 24 to 42 days. Blanckenhorn (2001) and Parker (1970) have also reported rates within this range. The egg to immature adult development rate (EggTime) in the model was therefore initially set at 33 days. Newly emerged adult *Scatophaga* flies will then undergo a period of feeding before becoming sexually active, which lasts approximately one and two weeks for males and females respectively (Blanckenhorn *et al.*, 2001). After considering the range of 8- 21 days reported by Gibbons (1980) and Parker (1970), the pre-oviposition period (ImmatureTime) in the model was initially set at 14 days.

Longevity

Scatophaga longevity was studied under laboratory conditions and the maximum longevity (MaxLifeSpan) was found to be equivalent to 71 to 97 days after taking account of the mean egg to breeding-adult duration (Jann & Ward, 1999; Martin *et al.*, 2004). In addition, Gibbons (1987) reported survivorship curves for *Scatophaga* generated using field-caught *Scatophaga*, showing percentage survival against the number of egg clutches the insects had laid. As field data were considered to be more realistic for this parameter, the maximum adult lifespan in the model was set to 80 days.

Fecundity

Females were allowed to lay 50 eggs per clutch (ClutchSize – the number of female eggs laid is 25, assuming equal numbers of male and female eggs are laid) in the model after Martin *et al.,* (2004) reported clutch sizes of approximately 40 eggs and Jann and Ward (1999) reported up to 60 with excess food. Gibbons (1987) found it took 6-7 days to mature another clutch of eggs under field conditions and so the time between clutches (ClutchTime) was set to 7 days in the model.

Survival Rates

Dung fauna toxicity tests only provide survival rates between hatched egg and newly emerged adult (and not for different larval instars or pupation). As a result the model assumes 100% survival for all ages within this range, with a single survival figure for the last age-stage corresponding to the test data. The survival from egg to immature adult determined in the control samples of the toxicity tests is used for the control populations in the model. In the model the survival from freshly laid egg to emergence is called SurvivalEggToImmature (Equation 4-4).

$$p_{EggTime+1} = SurvivalEggtoImmature \qquad \text{Equation 4-4}$$

This default value for the proportion of eggs to emerge successfully as adults is set to 0.82, the average proportion to emerge from the controls in the ring testing of the OECD guidelines (Rombke *et al.*, 2009).

The data from the survivorship curves presented in Gibbons (1987) (Figure 4-2) were used to estimate adult mortality rates from immature adults to MaxLifeSpan, using the times between clutches estimated above. Again, rather than interpolating a mortality rate for each day, the mortality rate was set to zero for every day except the last between each clutch, with the last day mortality based on Gibbons' survivorship curves.

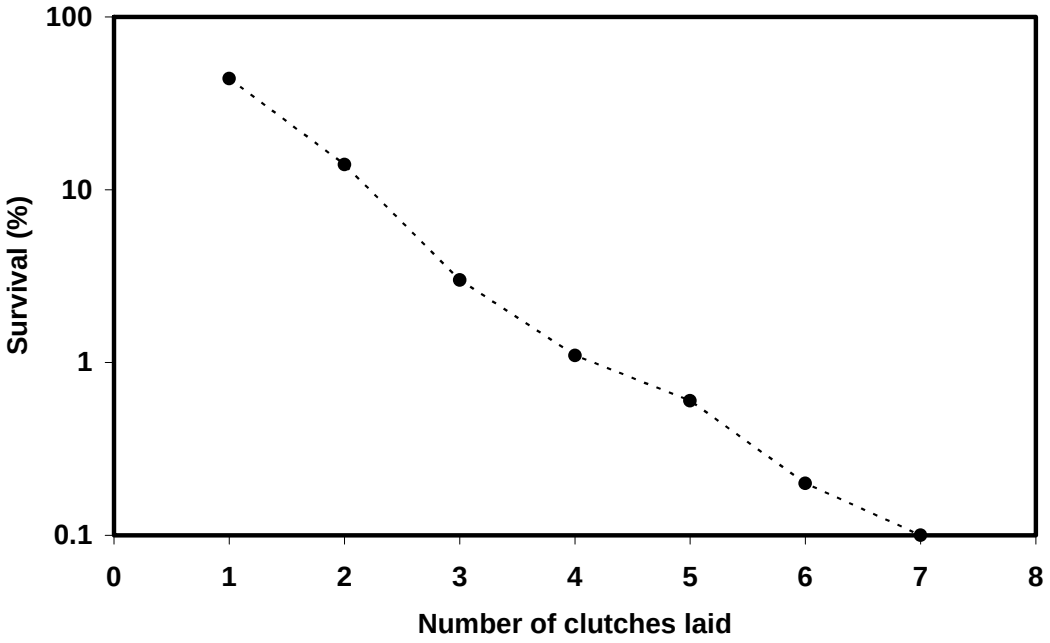


Figure 4-2 Adult *Scatophaga* survival rates estimated from Gibson (1987)

$$P_1 = \frac{Survival_1}{100} \qquad \text{Equation 4-5}$$

$$P_n = \frac{Survival_n}{Survival_{n-1}} \qquad \text{Equation 4-6}$$

Where $Survival_n$ is read from data presented in Gibbons (1987) for the n^{th} clutch and P_n is the value used in the Leslie matrix for p between the n^{th} and $n\text{-}1^{th}$ laying days. The values of P were calculated for each clutch. The mean P was 0.389 with upper and lower 95% confidence limits of 0.282 and 0.496 respectively. The mean value was subsequently used in the model as the adult survival between each ClutchTime interval.

Duration

The model duration is entirely based on the seasonality of the insect in question. Gibbons (1987) predicted *Scatophaga* begin emerging early in February following a field study undertaken near Durham, UK. As this is temperature

dependent it will vary greatly by location. The population decline at the end of the season, as the flies begin to overwinter as pupae, is not easily modelled without data to show how the appropriate parameters are affected. It was therefore decided to limit the duration of the model from insect emergence to the end of the autumn peak in field abundance (Duration).

Density Dependence

Amano (1983) and Sigurjonsdottir (1984) have demonstrated the effect of density dependence on the proportion of eggs within a pat to successfully emerge as adults.

In the model, survival to emergence with increasing density of individuals was modelled using data from Amano (1983) shown in Figure 4-3. If fly larvae require a certain mass of manure to complete their development then if there is more manure per larva available then we might expect density not to have an effect on the proportion to emerge. As the number of larvae per unit of dung increases there will be a point at which there is not enough dung for all larvae to complete their development and some will die (perhaps those with the slowest development rates). This is the point where density dependence affects the rate of emergence. A density independent value of 82% (Rombke *et al.*, 2009) appears to be consistent with the results of Amano (1983). At approximately 30 larvae per 50 g of manure, emergence is consistently below 82%.

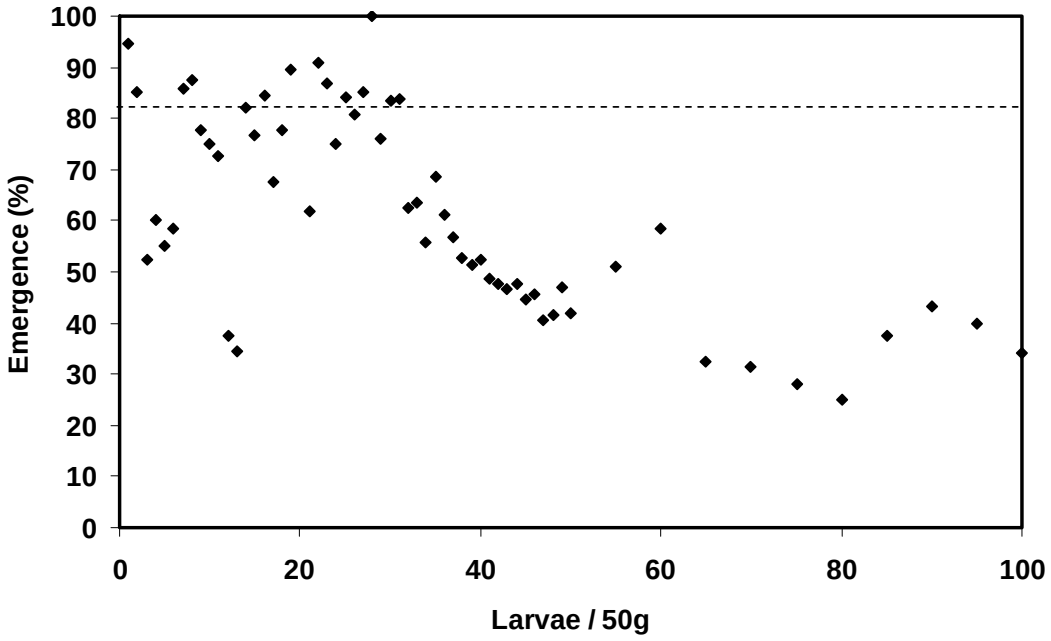


Figure 4-3 *Scatophaga* emergence at different larval densities (from Amano, 1983). The dashed line indicates 82%, the proportion to emerge from the control samples in Rombke *et al.*, (2009).

Assuming *Scatophaga* will only lay eggs in pats less that one day old, we can work out density dependence emergence over the whole field:

$$E = F \times S \times e \qquad \text{Equation 4-7}$$

Where E is the total manure (kg) produced on pasture per day, F is the Field site area (ha), S is the stocking density: number of animals per hectare and e is the manure produced per animal per day (kg).

For this model, the size of the pasture is 5 ha (the area of the pasture described in Chapter 2) and the maximum stocking density is 9.5 animals/ha (CVMP, 2008) leading to a maximum of 48 cattle (rounded up). Beef bullocks of approximately 500 kg body weight would produce approximately 9,185 kg manure per year (Spaepen *et al.*, 1997), resulting in 25 kg manure produced per animal per day. So, the 48 cattle in the pasture would be producing approximately 1,200 kg of manure per day.

The data from Amano (1983) were re-scaled from number of larvae per 50 g of manure to number of larvae per 1,200 kg of manure and re-scaled to allow 100% survival at the equivalent of 30 larvae/50 g to produce Figure 4-4. The equation from this fitted line was used to calculate the fraction of non-emergence due to the density of larvae (LarvalDensityFactor).

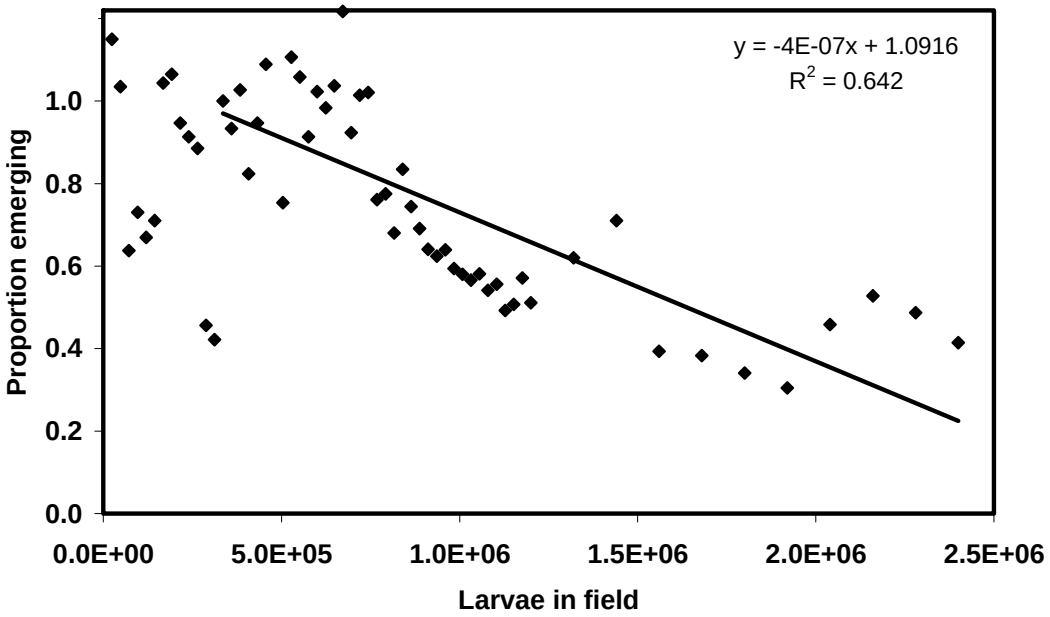


Figure 4-4 Density dependence of *Scatophaga* emergence in modelled pasture data from Amano (1983)

$$LarvalDensityFactor = 1.09 + -3.61E\text{-}07 \times E \qquad \text{Equation 4-8}$$

$$p_{EggTime+1} = SurvivalEggtoImmature \times LarvalDensityFactor \qquad \text{Equation 4-9}$$

Where E is the total number of eggs laid on the previous day.

LarvalDensityFactor was capped at 100% and 0% to prevent negative survival and more than 100% survival at very high larval densities and larval densities of less than 30 larvae/50 g dung, respectively. Key life history parameters used in the model are summarised in Table 4-3.

Table 4-3 Summary of model life-history parameters for *Scatophaga stercoraria*

Life-history parameter (time in days, survivals in proportion)	**Parameter name in model**	**Used in model**
Maximum lifespan (freshly laid egg to death)	MaxLifeSpan	80
Size of clutch (no. eggs per batch)	ClutchSize	25 (females only)
Time between clutches	ClutchTime	7
Time from egg to immature adult	EggTime	33
Time from immature to breeding adult	ImmatureTime	14
Egg to immature survival (at low densities)	$p_{EggTime+1}$	0.82
Adult survival between clutches	$\overline{P_n}$	0.389
Duration of the model (*Scatophaga* season from emergence to start of winter decline)	Duration	245

Seeding the Model

The model was initially seeded with 700 individuals normally distributed between 14 and 42 days old (n_{14} to n_{42}) and allowed to run for 5,000 days. The median total number of flies between n_1 and n_{48} over the last 100 days was approximately 1.4×10^8. The model was seeded with this median, normally distributed between ages n_1 and n_{48}, rounded to the nearest whole number. On running the model it was clear that this starting population was too high to replicate the pattern of growth observed by Gibbons and Parker (1987; 1970). After some exploration with different starting population sizes, an initial starting population of 570,000 was decided upon.

4.5.2 Case-Study: Ivermectin

The excretion data for the replicate time points were not normally distributed, probably due to the small number of replicates and high variability.

Consequently, the median of the replicates was used in the modelling. Modelling the excretion data was attempted using ModelMaker and using R (self-starting, first-order component model) but an acceptable fit could not be found. ModelMaker was found to grossly underestimate the peak levels measured on day three and the rate of decline and the R method could not work with the extreme values of day 3 at all.

The data were modelled in Microsoft Excel for the 25th, 50th (median) and 75th percentiles of the replicates of the measured data separately using the least squares method to optimise the fit. Although, as discussed in Chapter 3, it is possible that the day of peak concentration was missed due to insufficient sampling timepoints, this model initially assumes peak excretion to be on day 3. The data were split it into 2 parts: the increase in ivermectin (day 0 to day 3) and the decrease after reaching peak levels (day 3 to day 18). On modelling the medians, the initial increase was found to be nearly linear (R^2 = 0.97) and a power function fitted the data from day three satisfactorily (R^2 = 0.98). Figure 4-5 illustrates the modelled excretion data, and Table 4-4 summarises the model parameters.

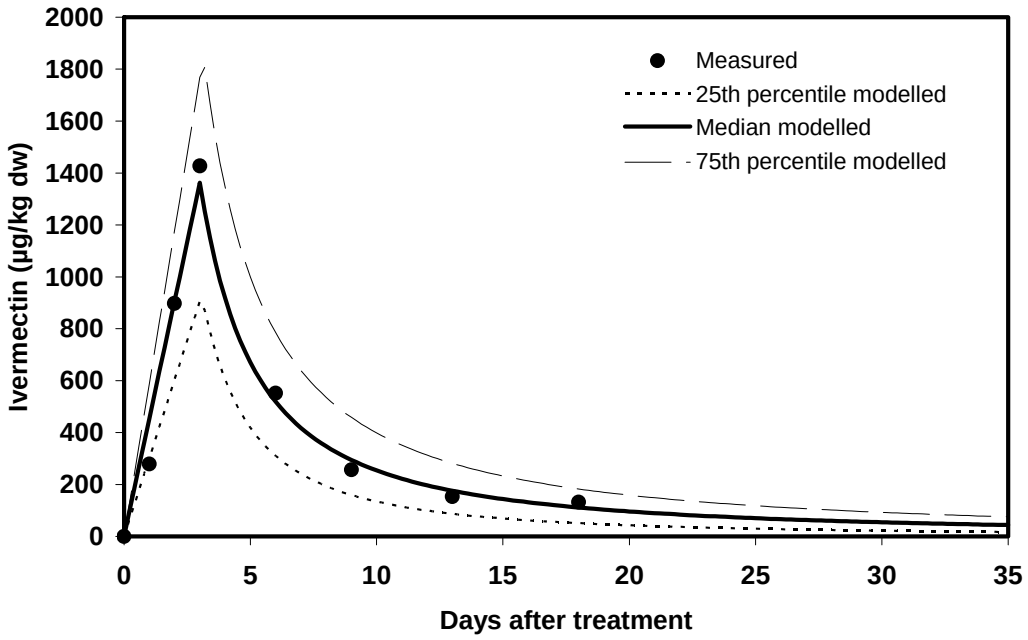


Figure 4-5 Ivermectin excretion following subcutaneous injection, modelled using Excel

Table 4-4 Summary of the modelled excretion data

		Lower limit (25% percentile)	Median (50% percentile)	Upper limit (75% percentile)
Days 0 – 3	Linear slope	301.89	454	589.43
Days 3 – 34	Coefficient	5853.3	6377.1	8447.8
	Exponent	-1.6389	-1.3995	-1.3260

Several authors have detected significant reductions in insect survival in dung collected beyond the 18 days sampled in this study (Kruger & Scholtz, 1995). This indicates that residues of ivermectin continue to be excreted after 18 days. The concentrations after day 18 were extrapolated from the modelled data up to day 34 following the bioassay results of Kadiri *et al.,* (1999) and Kruger and Scholtz (1995) who found significantly reduced emergence up to and beyond this period for *N. cornicina* and *Musca nevilli* respectively. Unfortunately, concentration data were not provided in these studies.

To determine whether the model used for the excretion profile is a reasonable one, the modelled excretion data were compared to previously published recovery studies. Firstly, the total dose administered was determined to be 60 mg per cow (assuming a body weight of 300 kg (based on the cattle actually used in this study) and a dose rate of 200 µg/kg body weight).

The next step was to calculate the total amount of ivermectin excreted within seven days to allow comparison with Chiu *et al.,* (1990). Using the same 300 kg body weight and daily dung production of 6% of body weight as wet weight of dung (Yan Zhixing from Merial, personal communication), it was calculated that a 300 kg cow would produce 18 kg of dung per day. Assuming freshly excreted manure is 85% water, the equivalent dry weight of manure per day is 2.7 kg. Using the modelled concentrations of ivermectin in dry weight of dung, the total amount of ivermectin excreted on a given day can be calculated. This information can be summed over the first seven days to give a cumulative total of ivermectin excreted over the first seven days.

Chiu *et al.* (1990) treated cattle with a radio-labelled subcutaneous treatment of ivermectin at a slightly higher dose rate of 0.3 mg/kg body weight. 62 ± 9.7% of the applied dose (i.e. the sum of ivermectin and metabolites) was recovered

during the first seven days after treatment, of which 39-78% was the original drug. This leads to an expected range of 20.4 – 55.9% of the total dose excreted as ivermectin over the seven days after treatment. The cumulative ivermectin excretion predicted by the excretion profile model used in the current study is 14.2 mg, or 24% of the applied dose of 60 mg, at the lower end but within the range determined by Chiu *et al.* (1990) and is presented in Figure 4-6.

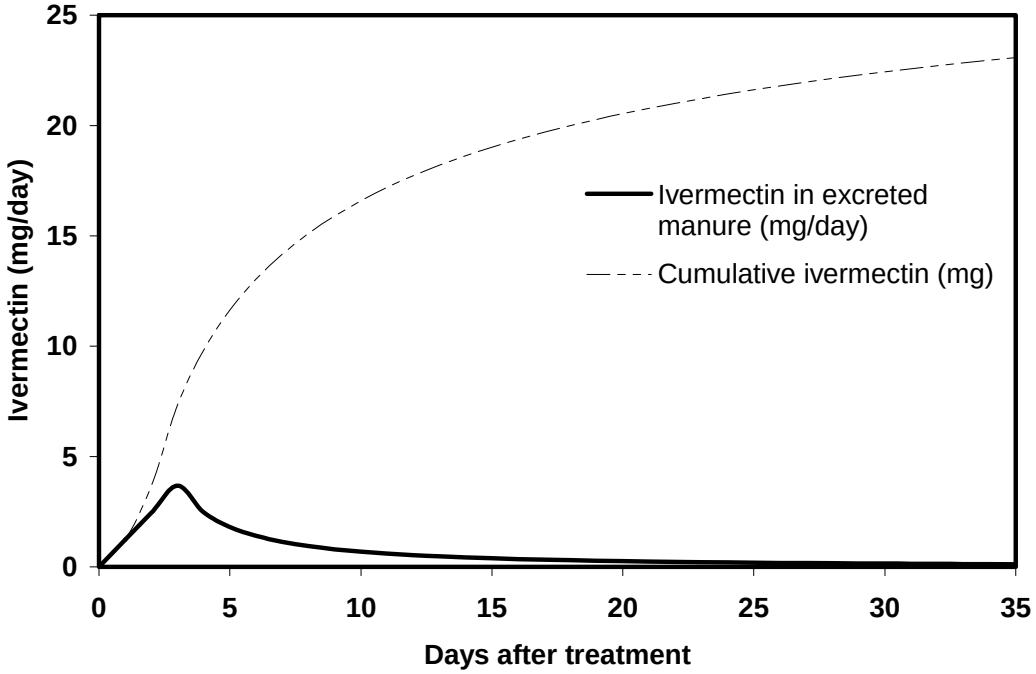


Figure 4-6 Modelled mass of ivermectin excreted per day and cumulative mass excreted

Comparable results were reported by Lumaret et al., (1993) where peak concentrations of 0.4 mg/kg were measured five days after a subcutaneous injection of ivermectin. If we assume the authors are referring to a wet weight concentration (the concentration is not reported as on either wet or dry weight basis) then the equivalent concentration would be approximately 2 mg/kg (dw), slightly higher than our results. Peak excretion five days after treatment is a longer lag time than in the current study but the article does not mention the variability in their measurement, if samples were taken in replicate, or if samples were taken from multiple cattle. Peak excretion was detected on the same day (3) after treatment by Iglesias et al., (2006), although significantly higher concentrations were reported, at 0.5ppm (dw) (equivalent to 0.5 mg/kg dw). The

variability in these measurements is also unclear as error bars are included on the graph but not described. Finally, no recoveries are provided for the analytical methods.

A vector called FieldConc was created to contain the field concentrations of ivermectin over the duration of the model (vector width = duration), initially comprised of zeros. The user inputs how many treatments there would be, and on which day the treatments start. The model then reads in the excretion text file containing the modelled excretion profile, overwriting the appropriate sections of the FieldConc vector with the excretion profile. This is possible due to only the current day's pats being attractive to the flies. Merial's recommendation that calves are treated 3, 8 and 13 weeks after turn-out (NOAH, 2008) equates to days 86, 121 and 156 in the model.

Dose-Response

The toxicity data for *Scatophaga stercoraria* were taken from Römbke *et al*. (2009), the results of the ring-testing of the new OECD guidelines on dung fauna testing. Concentrations were adjusted to dry weight equivalents assuming the dung consisted of 87% water and emergence corrected using Abbott's formula (Abbott, 1925). Normally, chemical exposure-response functions would be sigmoidal and monotonic (Bartell, 2007). In this case however, a sigmoidal curve could not be fitted to the data. This is likely due to one datum which appears to be an outlier. However, with so few data points it was difficult to justify the exclusion of this point. The fitted dose-response curve (Equation 4-10) used is shown in Figure 4-7 where Conc is the concentration on the day in the model when the eggs were laid (ng/g dry weight).

$$ToxMortality = A\,(1 - e^{(-C.Conc)}) \qquad \text{Equation 4-10}$$

The asymptotic curve shown in Equation 4-10 was fitted using the non-linear least squares method in R. The parameters A and C were found to be 108.2 and 0.004901 respectively. Dose-response data are usually analysed using probit analysis where the model returns an x value (e.g. concentration) for a specified y

value (e.g. 50% mortality). However, for this study it was desirable to use the full range of dose-response data so that the proportion of emergence could be calculated for any given parasiticide concentration, a more realistic approach given the range of concentrations expected in the field. The model described above returns an EC_{50} of 16.2 µg/kg (fw), very similar to the EC_{50} of 14.3 µg/kg (fw), calculated using the more conventional probit analysis by Römbke *et al.*, (2009).

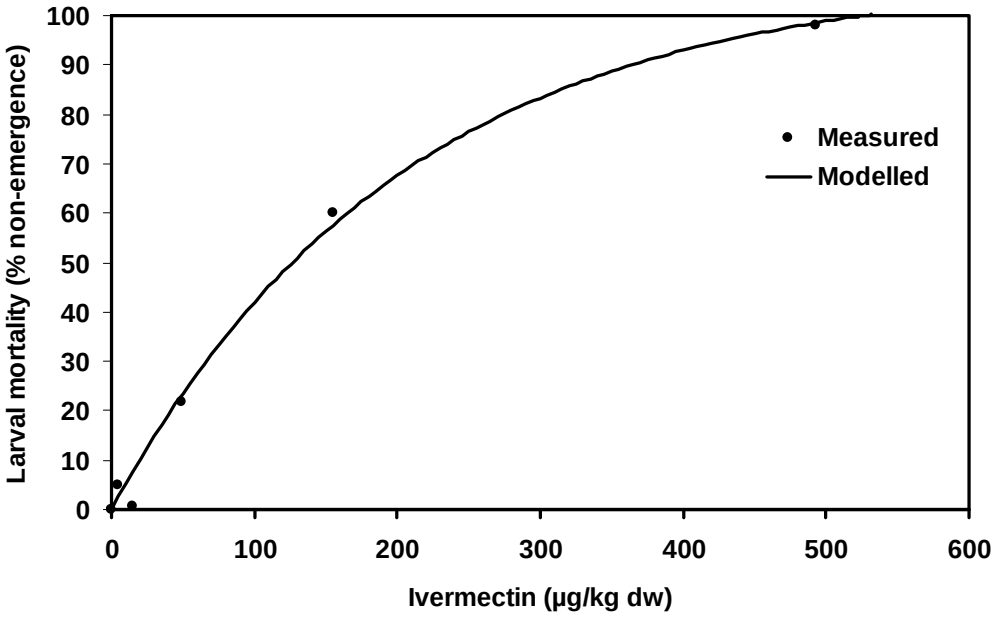


Figure 4-7 Dose-response curve for *Scatophaga* emergence with ivermectin concentration

A toxicity modifier (ToxMod) was then applied on calculating the survival to immature adult (n_{32} to n_{33}) according to Equations 4-11 and 4-12.

$$n_{ImmatureTime+EggTime} = p_{ImmatureTime+EggTime} \times ToxMod \times n_{ImmatureTime+EggTime-1}$$

Equation 4-11

Where:

$$ToxMod = (A \times (1- EXP (- C \times Conc)))/100$$ Equation 4-12

4.5.3 Analysis of Model Output

Due to the deterministic nature of the models, in both the density-independent and density-dependent models the first day the impact of parasiticide use is observed in the adult population will be 33 days after treatment, the egg to immature adult development time.

The impact metric used in the density-independent model is the mean percentage loss or decrease in abundance over the last fifty days (approximately one generation of the target organism). The percentage loss for each day is calculated according to Equation 4-13.

$$\%\ Loss = \frac{(C-T)}{C}.100 \qquad \text{Equation 4-13}$$

Where C is the number of adults (immature and breeding) in the control population and T is the number of adults in the treated population on that day.

It is not sensible to calculate the percentage decrease in abundance in the density-dependent model due to the variability in both control and treated population. Loss varies massively between days in the model. Using the median loss across a (variable) period of days was explored but was still found to be highly sensitive to the number of days used and when the period was situated in the model. In addition, the median treated population was in most cases statistically significantly higher than the control population (using the Mann-Whitney test). The impact metric used for the density-dependent model was therefore different: the number of days between the first day on which abundance declined after treatment, and the day at which the treated population once again recovered to match the control population.

Ideally, the pattern of simulated population changes over time predicted by this model would be compared to abundance data from field monitoring studies. Two such studies monitoring populations of *Scatophaga stercoraria* in UK pasture environments have been reported (Gibbons, 1987; Webb *et al.*, 2007).

Gibbons monitored Scatophaga numbers in Durham from April to December in 1964 and 1965 (1987). In 1965 populations were characterised by a spring peak which had finished by the start of June. Numbers of insects appeared to fluctuate considerably (between 600 and 6,000 daily catch). Numbers began to decrease by mid-October. In 1964, the population fluctuations are smaller, with daily catches between 600 and 2,000. A summer decline (diapause) was evident between mid-July and the end of September with numbers decreasing at the end of the year similarly to 1965. Several of the parameters used in the model developed in this chapter were taken from Gibbons, although not from this particular dataset. Compared to the results of the density-dependent model, insect emergence occurs at the same time, and the timepoint at which the model reaches its first peak and begins to fluctuate occurs about mid-May, broadly similar to that reported by Gibbons. That these dates are similar is particularly interesting since the density-dependent factors that drive this study's model were taken from a different source (Amano, 1983). That these data lead to similar results to those found in the field adds to the ecological relevance of the density dependent model. An attempt was made to model the diapause that *S. stercoraria* exhibit, but the model was not completed, and since the diapause is particular to *S. stercoraria* and not dung fauna in general, modelling the diapause was not considered a priority.

Webb *et al*., (2007) monitored *Scatophaga* adult abundance in cattle pastures in south-west Scotland. Abundance data are less detailed than that provided by Gibbons, as a single figure for the mean abundance for each month between April and July is presented during a two year study. Figures are provided for pastures grazed by cattle treated with ivermectin, doramectin and also untreated cattle. While the authors found that abundance data varied significantly between years and seasons, there was no significant difference in abundance between fields grazed by treated and untreated cattle. On a qualitative level, the density dependent model presented in this chapter also shows no apparent difference in *Scatophaga* population sizes between fields occupied by treated and untreated cattle, due to the density dependence parameters only affecting abundance when the population is un-stressed via ivermectin toxicity.

4.5.4 Sensitivity Analysis of Ecological Parameters

Sensitivity analyses on the model were carried out using the simple one-at-a-time approach (Dubus *et al.*, 2003). The input parameters were varied independently one at a time with other parameters kept constant and the influence on the model output observed. For the sensitivity analysis the parameters not under analysis were the default values detailed in Table 4-3 and the cattle were treated once, on the 22nd May. The variation in the ecological parameters used in the sensitivity analysis broadly represents the degree of variation reported for the individual parameters in the literature (see Table 4-2).

4.5.5 Model Exploration

The sensitivity of the model to changes to the case-study characteristics were explored by varying the toxicity of the compound and aspects of the exposure.

The sensitivity of the model to the compound toxicity was explored using the same one-at-a-time approach as described above for the ecological parameters. The changes to toxicity were achieved by varying the A parameter in the ToxMortality equation (Equation 4-10), by up to 20% in each direction.

The response of the population to changes in the experimentally-derived excretion data was examined using the 25th, 50th (median) and 75th percentile results, using the data illustrated in Figure 4-5 to generate different excretion profiles.

To explore the impact of specific changes to the excretion profile, such as the day concentrations of ivermectin reaches maximum, the magnitude of the peak and the rate of decline following the peak (k) the excretion data was modelled more simply using Excel according to Equations 4-14 and 4-15.

If t <= PeakDay then: $Conc_t = m \cdot t$ Equation 4-14

If t > PeakDay then: $Conc_t = PeakConc \cdot 10^{1-k \cdot (t-PeakDay)}$ Equation 4-15

Where t is the number of days after treatment, *PeakDay* is the number of days after treatment on which ivermectin concentrations reach their maximum (3), $Conc_t$ is the ivermectin concentration in manure in ng/g dry weight on day t, m is the slope of a linear line from zero to peak concentration (453.75), *PeakConc* is the maximum ivermectin concentration (1381 ng/g dw) and k is the rate of decline (0.23).

4.6 Results

4.6.1 Model Output

The population dynamics were simulated over 248 days, starting on the 26th February. Emergence of *Scatophaga* adults overwintering as pupae was set to peak on the 7th of March based on Gibbons (1987). Cattle treatment occurred on the 22nd May, 26th June and 31st July; 3, 8 and 13 weeks after cattle turnout on the 1st May. Figure 4-8 shows the model output without taking into account any density dependence.

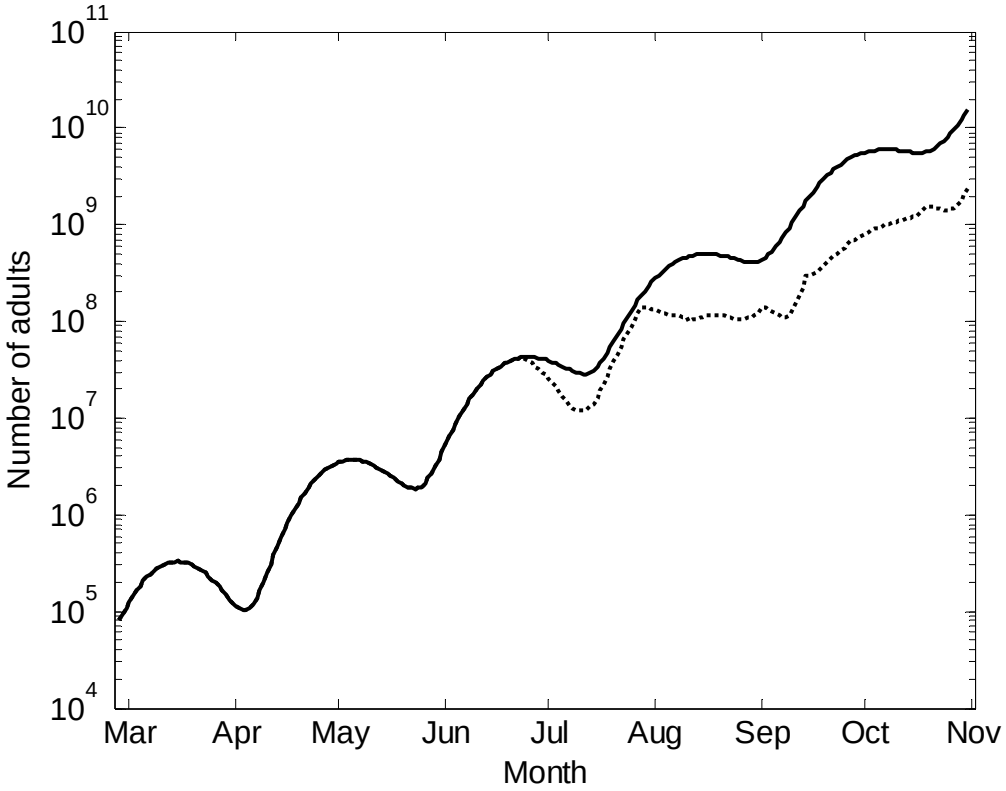


Figure 4-8 Cattle treatment on 22nd May, 26th June and 31st July, without density dependence. The dotted line represents the adult population in the pasture of treated cattle, the solid line represents the adult population in the pasture of untreated cattle.

The population in the untreated pasture increases in a series of bumps and troughs. These bumps correspond to the total development time from freshly laid egg to breeding adult. The effect of treatment is apparent in the number of adults from the 24th June, 33 days after treatment, the duration of egg to immature development. The mean decrease in the treated population compared to the control population over the last 50 days was 26% (with 95% confidence limits of 23 & 30). The maximum difference between the 'control' and the treated population was 88%, which occurred 197 days into the model (10th September), 41 days after the last treatment.

Figure 4-9 shows the model output incorporating density-dependence into the survival of egg to immature adult. The first effect on adult population abundance occurred again on the 24th June, 33 days after treatment. The number of adults from the treated pasture first matched the number of adults in the control

population (on simultaneous days) on the 23rd July (model day 148), 83 days after treatment after which the 'treated' population oscillates in the range of the control population oscillations.

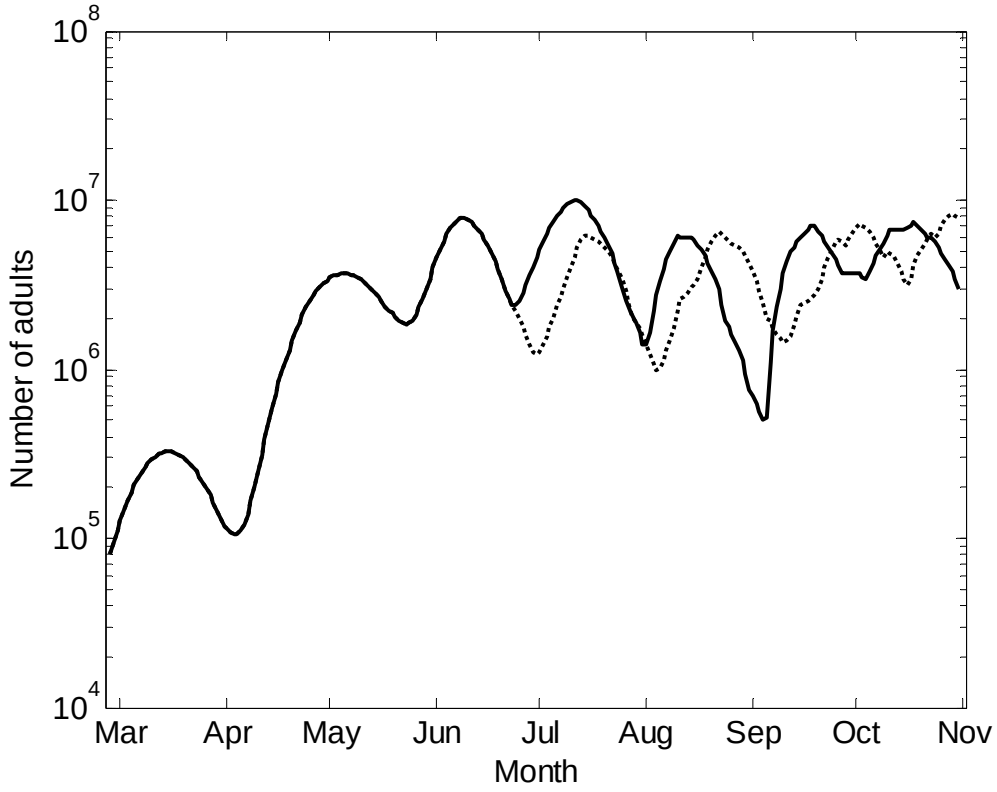


Figure 4-9 Treatments on 22nd May, 26th June and 31st July, with survival from egg to immature density dependent. Dotted line is the treated pasture, solid is untreated

4.6.2 Sensitivity Analysis of Ecological Parameters

The model was assessed in relation to the key ecological parameters. The results from the sensitivity analysis will be reported for the density-independent model followed by a summary of the density-dependent model.

The results from the sensitivity analysis for the ecological (life-history characteristics and seasonal) parameters are illustrated in Figure 4-10 for the density-independent model.

Fecundity: ClutchTime / Size

The model was relatively insensitive to changes in the fecundity parameters clutch size and clutch time. The loss in abundance (magnitude of effect) was

slightly higher when the clutch time was increased and the clutch size reduced. This means the effects of the ivermectin treatment were stronger when the fecundity parameters were reduced (insects reproducing more slowly).

Survival Rates

The model was similarly insensitive to changes in the survival parameters: egg to immature adult and immature adult to the maximum insect lifespan. A 20% increase in survival rates led to a small (2-3%) increase in ivermectin effect. An increase in survival led to an increase in the relative importance of the toxicity. As more insects survive to the next life-stage there are more survivors exposed to ivermectin. While a 20% reduction in egg to immature adult survival lead to a corresponding 2.3% reduction in impact, the same reduction in immature to lifespan survival lead to less than 1% decrease in impact. This may be expected as it is only the egg to immature life-stages that are exposed to the ivermectin as adult *Scatophaga* are predaceous and do not feed on manure.

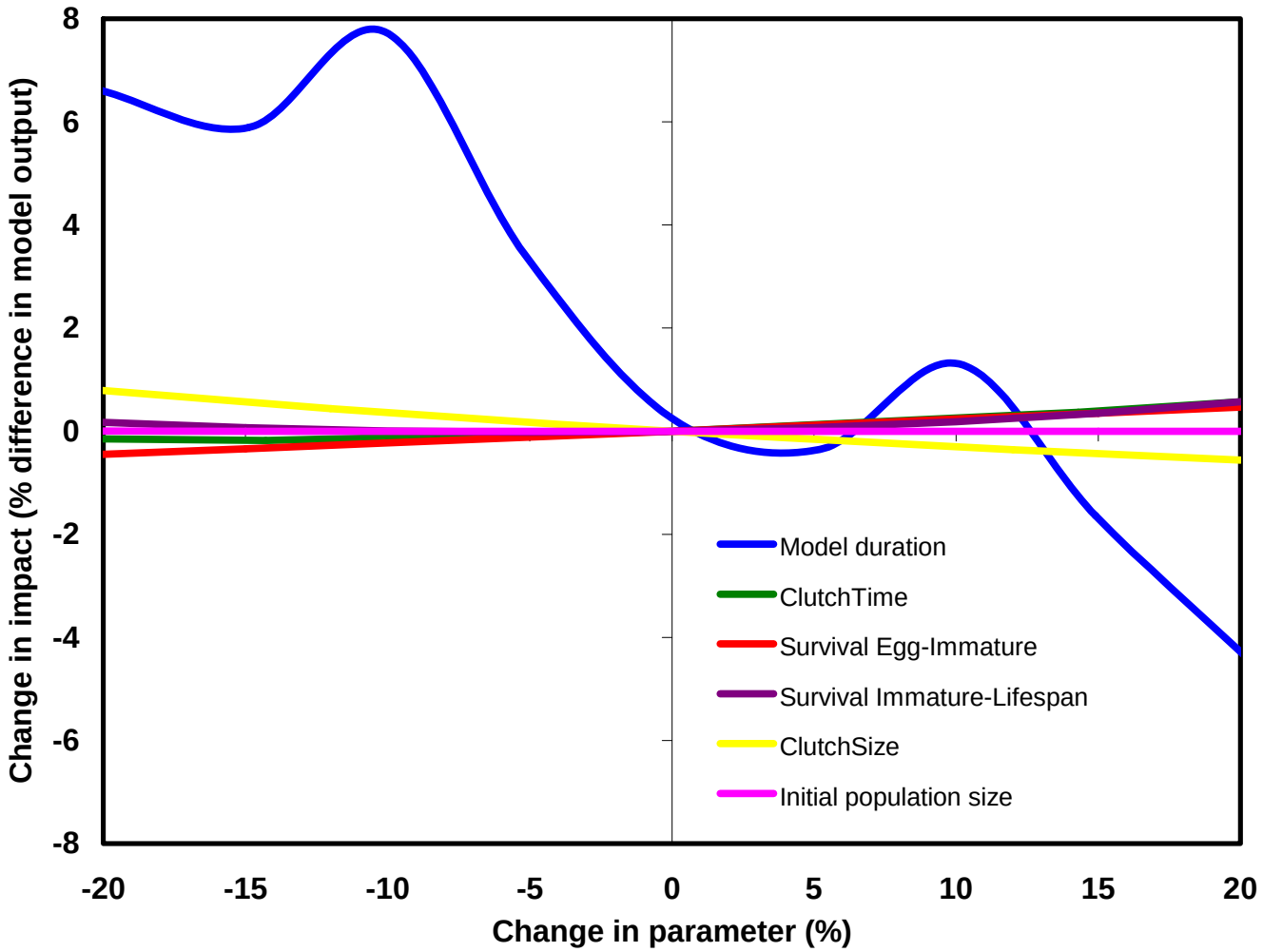


Figure 4-10 Density-independent model sensitivity to a range of ecological parameters

Model Duration (Response to Changes in Seasonality)

In the model, duration (the length of the *Scatophaga* flight season up to the start of the autumnal decline), affects the number of days the model runs after treatment. The model output was found to be more sensitive to changes in duration, with a twenty percent decrease in duration leading to up to an approximately eight percent increase in impact. Although the actual difference between the control and treated populations increase over time, the population growth reduces the relative importance of this difference, leading to a reduced impact.

Initial Population Size

Changes to the initial population size had no impact on the output from the density-independent model at all, as the relative population sizes remained the same.

Development Rates

The model was found to be highly sensitive to changes in the insect development rates, represented in the model as EggTime and ImmatureTime (Figure 4-11). The model was more sensitive to changes in egg-to-immature adult development time than immature-to-breeding adults as this was by far the longer development time. These parameters control the generation times and so the size of the generational 'bumps' seen in Figure 4-8. Changes in development rates therefore affect *when* in the generation treatment occurs (or *where* on the generation 'bump'). An increase or decrease in egg development time can lead to up to 30% more loss in abundance as shown by the parabolic shape in Figure 4-11.

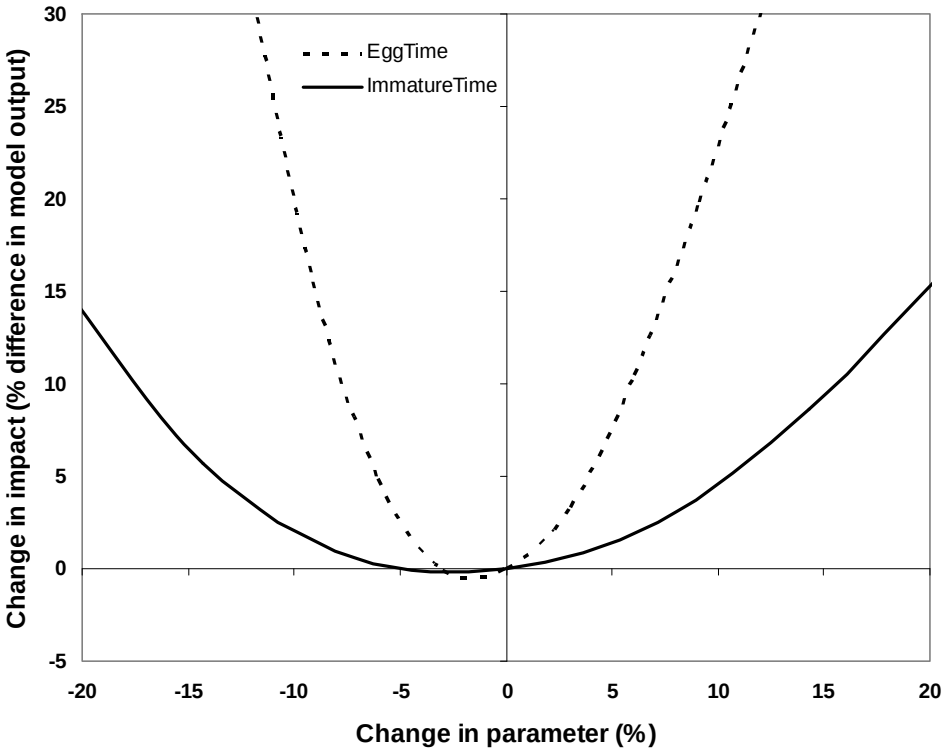


Figure 4-11 Density-independent model sensitivity to changes in development rates

4.6.3 Case-Study Scenario

Toxicity

Toxicity had the largest influence on model output, resulting in a clear positive effect on impact. A 20% increase and decrease in toxicity resulted in a 12% increase and 19% decrease in impact respectively.

Excretion Profile / Exposure

The sensitivity of the model to the range of experimentally-derived excretion data was explored, using the 25th, 50th (median) and 75th percentiles, and is illustrated in Figure 4-12 and summarised in Table 4-5. The excretion profile from the 25th percentile resulted in an 18% impact, a 31% decrease in impact compared to the median. The excretion profile from the 75th percentile resulted in a 33% impact, a 26% increase in impact compared to the median.

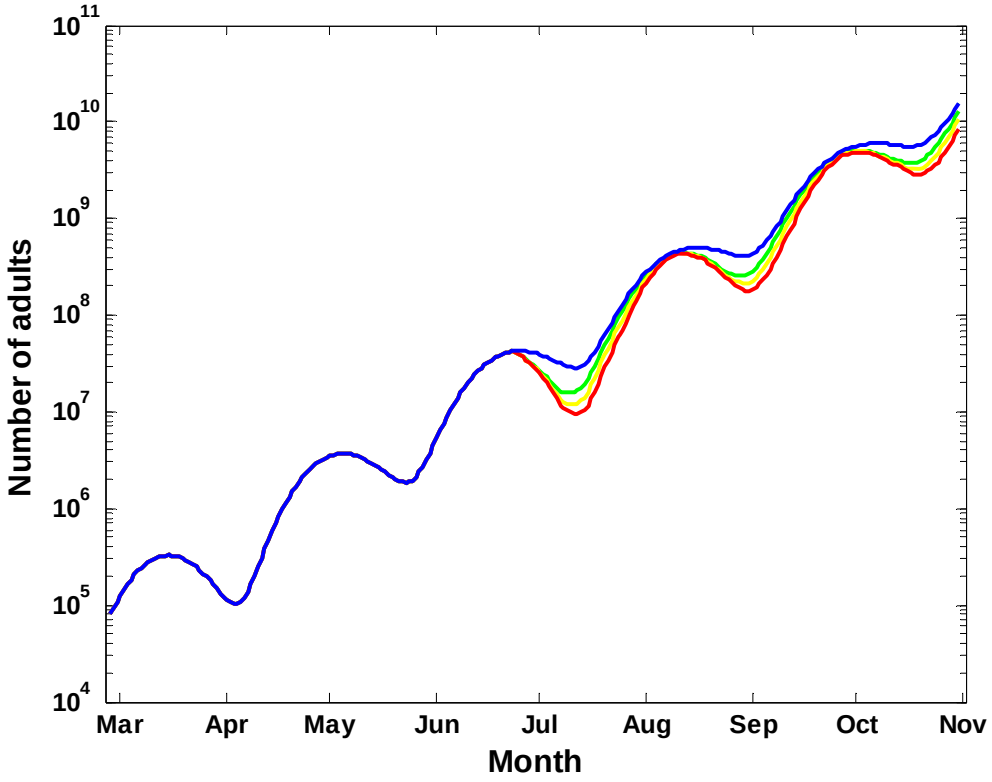


Figure 4-12 Model sensitivity to variation in measured ivermectin concentrations in excreted dung. Control in blue, 25th, 50th and 75th excretion data percentiles in green, yellow and red respectively.

Table 4-5 Effect of 25th and 75th exposure percentiles on predicted impact

	Exposure percentile	Reduction in abundance, compared to control	Influence on impact, compared to median
1 treatment	25th	18.0%	-31%
	median	26.0%	-
	75th	32.8	26%
3 treatments	25th	66.6	-20%
	median	83.2	-
	75th	91.9	10%

The sensitivity of the model to the exposure parameters was tested by varying the rate of decline following peak ivermectin concentration and varying the maximum concentrations measured at the peak as illustrated in Figure 4-13. The model was more sensitive to the rate of concentration decline than changes in peak concentration.

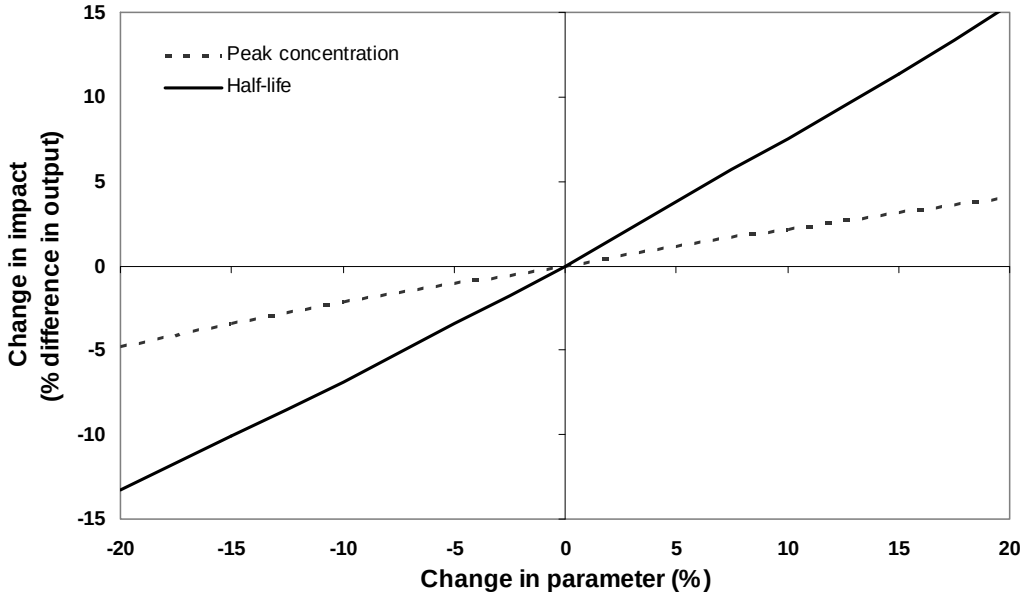


Figure 4-13 Density-independent model sensitivity to changes in exposure parameters where half-life is the rate of decline in concentration from the peak in excretion

The relative importance of the parameters described above are summarised in Table 4-6. The maximum impacts were all caused by the maximum change to the parameter except for the duration of the model.

Table 4-6 Index of maximum model sensitivity over 20% change in ecological, toxicity and fate parameters, calculated by dividing the change in output by the change in parameter multiplied by 100.

Parameter	**Sensitivity**	
Egg development time	2.63	Very high
Dose-response	0.93	Medium
Immature development time	0.79	
Duration of model	0.77	
Excretion half-life	0.77	
Excretion peak concentration	0.24	
Clutch size	0.04	Very low
Time between clutches	0.03	
Survival: immature → life-span	0.03	
Survival: egg → immature adult	0.02	
Initial population size	0.00	None

4.7 Discussion

4.7.1 Population Modelling to Refine Parasiticide Risk Assessment

If an 'unacceptable risk' is predicted for dung fauna at lower tiers of the tiered risk assessment for a new parasiticide, then further refinements are required in order to assess the impact under more realistic conditions. The lower tier approach is extremely simple, in the real world exposure will not be constant as assumed in the VICH guidelines (VICH, 2004), but will be pulsed, where incidence of exposure will depend on the treatment regime (e.g. application recommendations). Concentrations of the parasiticide in the field will also vary depending on the pharmacokinetics of the compound (e.g. excretion rate, degree of metabolism) in the treated animal and the fate of the compound in the pasture (e.g. persistence). The magnitude of the impact of the parasiticides will not only be influenced by the inherent toxicity of the parasiticide but will also be affected by the insect of interest's ecology, its life-history characteristics and the overlap of its seasonal activity period with field residues. Whilst field-based studies offer one approach to better assess risks in the real environment, these studies can be costly, time consuming and may not cover the time period of interest.

A population model was therefore proposed to help refine the risk assessment considering changes in exposure, insect ecology and treatment practices. The proposed approach assesses the impact of ivermectin on dung fauna using the information that would be available as part of the package of information for new compounds and existing information on dung insect ecology. A matrix population model was developed to predict the impact of treating pastured cattle to a range of dung fauna using insect phenologies, life-history characteristics and survival rates in the absence of parasiticide exposure from the literature. The response of the population was expressed as the percentage loss in the adult abundance in the pastures of treated cattle compared to a pasture of untreated cattle. Use of the model was demonstrated using a case-study, modelling the response of a population of *Scatophaga stercoraria* to ivermectin treatment.

4.7.2 Case-Study Scenario: Response of Population of *Scatophaga Stercoraria* to Ivermectin

In the case-study, it was assumed the cattle were treated with a subcutaneous injection formulation of ivermectin 3, 8 and 13 weeks after being turned out to pasture on the first of May, based on the recommended dosing regimes from the parasiticide supplier. Exposure was estimated from the field studies reported in Chapter 3 and the cattle treatment regime.

The difference in the predicted impacts between the density independent and the density dependent models was very large. In the density-independent simulation the estimated loss in adult abundance was 87%, with exposure 25th and 75th percentiles returning a loss in abundance of 67 and 92%. In the density-dependent simulation, whilst the degree of departure from the control did vary with the median, 25th or 75th percentile exposure, the day on which the 'treated population' first matched that of the control population (the recovery period) was the 23rd July (model day 148), 83 days after the last treatment, the same day for each.

The density-dependent simulation demonstrates that an insect species with a high reproductive capacity, such as *S. stercoraria* has the potential to rapidly recover from the parasiticide-induced losses. These model results are supported by the findings of the field monitoring study of Webb *et al*., (2007) in south-west Scotland. In this study, cattle were treated with two doses of either ivermectin or doramectin, at cattle turn-out and 8 weeks later. *S. stercoraria* abundance was monitored between April to July using dung baited pitfall traps. No difference in abundance between the control pastures or the treated pasture could be attributed to avermectin use.

4.7.3 Model Evaluation

Sensitivity analysis in the form of the one-at-a-time approach (Dubus *et al.*, 2003) was employed throughout model development. Once the development of the density-independent model was completed, the influence of the parameters

on the model was assessed both in terms of the impact of the case-study scenario and for application of the model to a theoretical species model.

As the impact is measured by comparing the abundance in the 'treated' population to that of the 'control' population; the fecundity parameters (clutch size and time between clutches), base survival rates and initial population size had very minor or no influence on the model output. For this scenario the value for these parameters were selected either from the range of data reported in the literature as in the case of the fecundity parameters or from the results contributing to the ring-testing of the new OECD dung fly testing guidelines (Rombke *et al.*, 2009). It would therefore seem reasonable to make these parameters constants in a model examining the impact of parasiticides on theoretical dung fauna. If however, the actual abundances were required, for example to compare the abundance of one species to another, then these parameters should be adjusted accordingly.

The model was found to be particularly sensitive to changes in insect development rates, toxicity, model duration and exposure to residues. Insect development rates are temperature dependent (Blanckenhorn, 1997) and will therefore vary from site to site and within the year. Broadly speaking, development rates tend to be slower during the colder months. However, in the case of *Scatophaga stercoraria* there is the further complication of the summer diapause when field abundance rapidly declines (Parker, 1970; Gibbons, 1987; Blanckenhorn *et al.*, 2001). For a more realistic *Scatophaga* model it should be possible to incorporate this diapause into the model, by adjusting the fecundity parameters over the summer period. This was attempted in the density-dependent model but not adequately accomplished. As the aim of the model was to develop an example for modelling the impact of parasiticides on a range of dung fauna and not for *Scatophaga* alone, incorporating the summer diapause was not deemed a priority.

Toxicity and exposure were understandably very important in predicting the impact of parasiticide use on the insect population. The determination of the excretion profile during ADME studies is performed under highly controlled

conditions and relatively precise measurements are reported. However, this may not be representative of the situation under field conditions where livestock are unlikely to be weighed prior to treatment and their diet not closely monitored. Given the large influence the exposure parameters have had upon the model output it may be sensible to allow for likely variation in these results, using the range of data generated from the excretion study to predict the range of population-level response.

Varying the toxicity of the modelled compound had a relatively large influence on the change in insect abundance. A ten percent decrease in toxicity reduced the impact on abundance by nine percent. Traditional endpoints such as NOEC's or EC_{50}'s do not inform us of the rate of increased effect with increasing exposure. In this model the full dose-response relationship was used in order to best estimate the most realistic range of effects associated with real exposure levels.

The exposure component of the model was assessed using the range of data determined in the excretion study and by varying the peak concentration and the elimination half-life from a simplified excretion profile model. The *Scatophaga* model was much more sensitive to changes in the elimination rate and length of time during which residues reach the field than to changes in the peak concentration. Results would suggest that formulations excreted more rapidly but with a higher peak levels such as the pour-on have less of an impact on *Scatophaga* populations than the injection modelled here.

4.7.4 Incorporating Stochasticity

Incorporating stochasticity into the model was explored but ultimately rejected. The model was run a large number of times with different parameters for all the individuals for a given run. While this introduced variations between results of different runs of the model, it is not true stochasticity. To be truly stochastic the model would have to show variation between different individuals, reflecting the variation observed in the field or laboratory. Due to the nature of the age-structured matrix model, the matrix is used once a day to determine the numbers

in each population class for the next day from those of any given day. One approach to introduce a measure of stochasticity to the model would be to vary the non-zero elements (e.g. survival and fecundity rates) in the model between iterations according to a normal distribution. While this model would no longer be deterministic, varying parameters in this fashion would mean that all individuals on a given day have a different mortality or fecundity than on the previous day, but all still have the same fecundity on any given day. In addition, sensitivity analyses have shown these parameters (survival rates, fecundities) have very little effect on the model output (expressed in terms of a comparison of abundance in 'treated' populations compared to the 'control'), making these parameters stochastic therefore will add little value to the model. In contrast, the model output is very sensitive to changes in development rates so making these parameters stochastic really would add value. However, adding stochasticity to the development rates would be more difficult as it affects the size of the matrix, which as explained, is defined at the start of each model simulation. If a probabilistic model is desired then a different modelling approach could be employed, such as an individual-based model (IBM) which is better suited to producing probabilistic results.

4.7.5 Inclusion of Density Dependence

In the field, the population size limit is likely to be controlled by a number of factors. Climate conditions such as high temperatures have been demonstrated to cause a reduction in reproductive output and this has been linked to the decline in adult *Scatophaga* in mid-summer (the diapause) observed in the field (Blanckenhorn *et al.*, 2001; Gibbons, 1987; Parker, 1970). Other factors may include intra-specific competition among adult flies for prey and inter-specific competition for space and food for larvae developing in the pat (Hirschberger & Degro, 1996). While several of the parameters may be altered according to density there are few published studies to draw on for data. However, there is clear evidence for density dependence operating on the proportion of larvae emerging from the pat (Sigurjonsdottir, 1984; Amano, 1983) and so this aspect was incorporated into the model.

Visual inspection of the output from the density-dependent model (Figure 4-9) demonstrates the potential usefulness of this approach. Incorporating a density-dependent aspect into the model means that the capacity for a population to recover or compensate may be explored. However, establishing a useful numerical endpoint was problematic. A number of approaches to analysing the results were explored and ultimately rejected as sensitivity analysis highlighted major flaws. On reaching the carrying capacity (controlled by the weight of manure produced per day) the control population falls as the number of insects surviving in the crowded cow-pat declines. The adult population then continues to fluctuate in a chaotic fashion for the duration of the study. This is reasonable due to the lag time in the population dynamics when a population responds to density in one life-stage and reproduces in a later one (Begon & Mortimer, 1986). If the simulation was continued for long enough the fluctuations stabilise into repeating patterns (every 96 days) between 1.6×10^6 and 5.7×10^6 (with a median of 3.8×10^6). The large fluctuations within the normal model duration however made comparing the control and treated populations problematic. The populations were compared using non-parametric tests due to the non-normal distribution of the populations. The Mann-Whitney test showed the control and treated populations were almost always (after varying the parameters) statistically different, but a higher median was usually observed in the treated. This is partly because the control population shows a characteristic drop in numbers around the start of September and after an approximately 40 days delay the treated population drops in a similar fashion, which was outside of the normal duration of the model. The length of this delayed drop varied considerably with small changes to the insect development rates amongst other parameters.

Other possible endpoints associated with abundance were explored such as making development rates stochastic (but see problems outlined above), running the model many times and comparing the resulting sets of populations, and simpler approaches such as the first day the control and treated populations overlapped after treatment. These approaches were rejected after sensitivity analysis showed them to be far too sensitive to small changes in several of the input parameters.

The exclusion of density-dependence means that the potential impact of ivermectin may be over estimated, by eliminating the population's potential to recover or compensate by increasing survivorship as parasiticide-induced mortality reduces competition for available resources (here the manure). For a conservative assessment of the impact, results from the density-independent model may be satisfactory. Barnthouse *et al.,* (2007) remarks that it is not always necessary to build density-dependence into risk assessment models, provided that the prediction horizon is short and the population changes modelled are relatively small. However, incorporation of density-dependence for slow breeding insects where model projections are more long term may be more useful since projections from density-independent models will either grow to infinite size or decline to zero.

Density-independent models for populations that do have density-dependent regulation may be misleading (Moe, 2008). As field studies of *Scatophaga* abundance also show chaotically fluctuating populations around what may be the population's carrying capacity (Parker, 1970; Gibbons, 1987), risk assessment may be improved by using models which incorporate density-dependence. However, it may be more useful to use different endpoints such as the equilibrium density which tends to be lower in toxicant stressed populations (Moe, 2008).

4.7.6 Recommendations

There are a number of ways this modelling approach could be further developed to improve the predictions of real-world impact of parasiticides on dung fauna. Firstly, the collation of ecological characteristics of real dung fauna to develop a number of theoretical dung insect species, representing the range of diverse characteristics observed in the dung fauna community. Table 4-7 gives an example of three theoretical species, a fast breeding fly with parameters based on *Scatophaga stercoraria*, a univoltine beetle and a multivoltine beetle, taken from Wardhaugh *et al* (1998; 2001a).

Table 4-7 Model insect examples, beetle parameters are taken from Wardhaugh et al., (1998; 2001a)

Model insect	Egg to immature adult development time (days)	Pre-reproductive period (days)	Fecundity	Adult female survival
Fast breeding fly, e.g. *Scatophaga stercoraria*	33 (24 – 42) (Strong & James, 1992)	14 (8 – 21)	50 eggs per clutch, 6- 7 days to produce new clutch (Gibbons, 1987)	Half-life of ~ 4 days (inferred from Gibbons, 1987)
Multivoltine beetle (multiple generations per year) e.g. *Onthophagus taurus*	40 (30-50)	10	2 eggs per day over 6 weeks	Half-life of 4 weeks
Univoltine beetle (one generation per year) e.g. *Onitis alexis*	80 (60-100)	10	2 eggs per day over 10 weeks	Half-life of 8 weeks

The effect of different life-history characteristics on population responses have been demonstrated in the field and in computer simulations. Beketov *et al.*, investigated the effect of different life-history characteristics on the recovery rates of different invertebrates in a simulated stream system following contamination with the insecticide thiacloprid (Beketov *et al.*, 2008). Principal response curve analysis was performed for the short-lived, multivoltine macroinvertebrates (11 species) and the longer-lived, semivoltine macroinvertebrates (10 species) separately. The response in the shorter-lived species was severe and rapid, with population recovery within 10 weeks. The response in longer-lived species was slower, with numbers still declining 27 weeks after exposure and no recovery observed within the duration of the study.

The matrix population model described here may be easily applied to a range of dung fauna species with a range of life-cycle characteristics and phenologies. Our matrix model is designed to easily include the additional effects data describing how parasiticides may affect dung fauna, and these data could be measured in the testing of dung beetles. The guidelines for these studies are currently under development by the DOTTS group but due to the nature of the tests (the beetles will be introduced as adults and subsequent reproduction rates will be monitored) endpoints may include adult mortality and development rates as well as the proportion of insects to successfully emerge as adults.

Another useful addition would be to include other environmental stressors such as drought. A population already experiencing stress from other sources may be more vulnerable to the effects of a parasiticide. There is some evidence for this from the field studies conducted over two years in South Africa by Kruger and Scholtz (1998a; 1998b) where a relationship between the abundance, diversity and biomass of the insects collected within pats was observed only in the year experiencing drought. The combined response of the population to these additional stressors may be additive or even synergistic, greatly influencing the predicted risk to the population.

Other useful aspects to consider include the effect of the proportion of the cattle treated or the availability of manure from untreated cattle, as the effect of a toxicant on local populations could be dampened by migration from healthy individuals into the exposed environment (Ares, 2003). One way of exploring the mitigating effects of nearby sources of 'untreated' manure is by introducing a spatial element to the model (e.g Vale & Grant, 2002), incorporating insect dispersal rates and distances. Webb et al., (2007) used the dispersal distances measured for blow flies as an example of potential *Scatophaga* transport. Roslin (2000) investigated the movement of *Aphodius* beetles at two spatial scales, between pats within a pasture and between pastures. Roslin found a power function to adequately describe the distribution of dispersal rates with distance and when comparing the dispersal patterns of different *Aphodius* species found movement between pastures was more frequent the larger the species, the more specific it is in its preference of pat age (position in the successional colonisation of pats) (Roslin, 2000). Inclusion of these factors was unfortunately not possible within the duration of this project.

4.8 Conclusions

This chapter has investigated the hypothesis that a simple Leslie matrix model could be used with available information to assess risks to dung fauna in a real-world setting where tier one assessments dictate that further investigation is required. Due to time constraints the model was not sufficiently developed to fully prove the hypothesis. Selecting a reasonable endpoint of the density-dependent model was particularly difficult and time-consuming, which made it impossible to accurately evaluate that model. Since the matrix varied over time due to the inclusion of excretion profile and treatment regime data, some of the benefits of an age-structured model were lost. In hindsight, an individual based model may have been better suited to this scenario.

The model as presented shows that it is possible to include excretion rate data such as that gathered during the fate and inputs assessment described in Chapter 3 to build a population model for the purposes of risk assessment. However, it appears that the Leslie matrix method is not well suited to this purpose as the

varying nature of the matrix over time once the excretion and treatment regime are included leads to results that are very difficult to assess. To accurately reflect the field conditions, density dependence should be included in the model. However, the density dependent model as presented here would be very sensitive to the survivorship figure chosen for the proportion of eggs that emerge as adults. The value used in the model (0.82) was based upon laboratory studies which do not adequately reflect competition and other environmental effects, and as a result was likely to be unrealistically high. However, since a more realistic figure for this value under field conditions was not even available in the literature for *Scatophaga,* a well-studied insect, it must be estimated by experienced ecologists who better understand the nature of the insects being modelled, whether modelling an actual or a hypothetical species.

Numerous studies have demonstrated that parasiticides may have an adverse effect on pasture fauna. What is less clear is the extent to which dung fauna populations are affected. For the first time a matrix population model was developed for dung fauna (using *Scatophaga stercoraria* as an example) using the information that would be available for the registration of a new parasiticide; life-history and phenology information in the literature, results from laboratory toxicity tests (new OECD test 228) and excretion data. In a density-independent scenario where pastured cattle were treated 3, 8 and 13 weeks after cattle turn-out the predicted loss in abundance was 83%. This conservative estimate may be further refined incorporating density dependent factors, potentially allowing for the population to recover after cattle treatment. For a Leslie matrix based approach to be useful, further work is required to determine more realistic input data and explore other endpoints such as recovery time, probability of extinction and equilibrium (steady state) abundance for density dependent models.

Extension of this model to other dung fauna species would enable a comparison of likely impacts on species with differing life-history characteristics, such as multivoltine and univoltine dung beetles. Finding a complete set of life-history parameters for other species may not be possible without further species specific studies. One solution is to develop theoretical insect species based on insects with similar ecologies (e.g. Wardhaugh *et al.*, 1998; Wardhaugh *et al.*, 2001a;

Vale & Grant, 2002). Furthermore, exposure scenarios based on local husbandry and treatment regimes may be developed.

Matrix models are easy to use and intuitive and, while software is available (e.g. RAMAS GIS), it is also possible to write the model using a program such as Matlab or R. It has been demonstrated that applying matrix population models to estimate the impact of parasiticide use is potentially a useful approach for assessing the risk posed to dung fauna exposed to parasiticides. However, further work is required to develop the model presented into a usable solution, or alternatively to include the excretion rate data into an individual based model. Modelling could be cost-effective for risk assessment before an expensive and logistically-difficult field study is undertaken, or perhaps as an intermediate step. Suggestions have been made to further develop the modelling method proposed here to further refine the risk assessment for parasiticide use on dung fauna.

5 Discussion

The field studies included in this thesis and other studies have demonstrated that parasiticides are released into the environment following the treatment of pastured livestock (Herd *et al.*, 1996; Erzen *et al.*, 2005; Suarez *et al.*, 2009). Due to their mode of action, many parasiticides are highly toxic to insect species and once released into the environment they have the potential to have an adverse effect on fly and beetle populations. It is therefore important that the potential effects of parasiticides on non-target insect species be assessed before parasiticide use is authorised. The environmental safety of new parasiticides must therefore be demonstrated by undertaking a risk assessment before they are given market approval. The VICH guidelines and the supporting EMEA guidance document provide advice for performing the initial stages of the risk assessment, but this advice is limited with respect to methodologies for refining the risk assessments if an 'unacceptable risk' is determined for dung fauna using the more conservative assessment procedures. The aim of this thesis was therefore to develop an improved understanding of those factors and processes affecting the risks which parasiticides pose in the pasture environment, with a view to developing improved methods for assessing the environmental risks of parasiticides. In this section, the results of the experimental and modelling chapters are combined to a) assess the risks of ivermectin use in the pasture; b) to explore the wider implications of the work for the environmental risk assessment of parasiticides; and c) to identify major knowledge gaps that remain and recommend priorities for future research.

5.1 Risks from Ivermectin in the Terrestrial Environment

This study has clearly demonstrated that ivermectin is excreted by treated livestock into the environment, confirming the reports by other authors (e.g. Herd *et al.*, 1996). In addition to the parent compound, for the first time two metabolites were identified in the excreted manure; namely the 24-hydroxylmethyl H_2B1_a and 3"-O-Desmethyl H_2B1_a both derivatives of ivermectin. These metabolites may also have anti-parasitic properties as

functional groups (namely the sugar group and a hydroxyl group) key to the activity of the parent compound are still intact (Fisher & Mrozik, 1989; Shoop & Soll, 2002). This demonstrates the importance of considering the risks of not only the parent compound but also the metabolites in the risk assessment process.

Excreted ivermectin was found to be persistent in the UK pasture environment, with no significant change in concentrations observed within the life-time of the pats (>38 days). Comparable results indicating the high persistence of ivermectin residues in manure were reported by Sommer *et al.,* (1993) in Denmark where no change in concentration was observed in the study time period of 45 days. Two field studies performed in Argentina, one in the autumn and one in late spring, also found no change in concentration after 60 and 180 days respectively (Iglesias *et al.*, 2006; Suarez *et al.*, 2003).

Ivermectin residues were demonstrated to transfer to the soil directly beneath the pat, with the highest concentrations measured after the pats had degraded in the field, at mean concentrations of 56 µg/kg (ww) and 12 µg/kg (ww) in the 0-1 cm and 1-3 cm depths respectively. Although the method for analysing ivermectin residues in soil used in this experiment has limitations for low concentrations, at the peak concentrations found in this field study, the method is acceptable and therefore these conclusions are reliable. Ivermectin has previously been shown to degrade slowly in soil with half-lives of between 21 and 56 days reported in soils (Bull *et al.*, 1984), and a series of lab studies using soil from the same site as this field study reported a DT_{50} of 67 days at 20ºC (Krogh *et al.*, 2009). Ivermectin residues may therefore have the potential to build up in the soil following repeated exposure, such as repeated use of the same pasture by ivermectin-treated cattle. This may result in long-term exposure of soil organisms to ivermectin residues.

Tables 5-1 and 5-2 summarise the results of a risk assessment for soil and dung fauna using the methods described in the guidelines (VICH, 2000; VICH, 2004; CVMP, 2008). In each table, successive tiers correspond to increasing levels of refinement, for example lower tiers use highly conservative, worst case estimates

of concentrations, while higher tiers use more environmentally relevant measured concentrations.

In Phase I of the risk assessment process, the predicted environmental concentration for ivermectin in soil (PEC_{soil}) is calculated using the method described for pastured livestock in the EMEA guidance document (CVMP 2008) (Table 5-1), using the default values given for stocking density, soil bulk density and assuming a 5cm mixing depth. These calculations result in a PEC_{soil} of 0.836 µg/kg. This is well below the action limit of 100 µg/kg (dw) set in the VICH Guidance for Phase II assessments on soil (VICH 2004), so for certain veterinary medicines the risk assessment process would stop here.

This study has generated a measured environment concentration (MEC) for ivermectin in the top 1cm of soil, directly under a pat. When this value is used as a Phase II refinement, the resulting risk quotient is greater than one (Table 5-1), signalling a potential impact on soil fauna. However a number of mitigating factors should be considered when interpreting this higher risk, as it is only in the top centimetre of soil directly under pats and not evenly distributed across the pasture, i.e. in isolated hotspots.

Due to their known potency to insects, when assessing the impact of parasiticides to be used for pastured livestock, Phase II studies on dung fauna are triggered regardless of the results of Phase I assessment.

Table 5-1 Summary of the risk characterisation calculated using CVMP 2008 guidelines for soil organisms

	PEC (mg/kg dw)	**Toxicity (mg/kg dw)**	**Assessment factor**	**PNEC (mg/kg dw)**	**RQ (PEC/PNEC ratio)**
Phase I	0.000836*	*F. fimetaria* NOEC: 0.3 Jensen *et al.,* (2003)	10	0.03	0.028
Phase II	0.062 (estimated from the MEC of 56 µg/kg ww, assuming a water content of 11%)	*F. fimetaria* NOEC: 0.3 Jensen *et al.,* (2003)	10	0.03	**2.07**

*calculated using recommended ivermectin treatment regimes (NOAH 2008) and default values from CVMP, 2008

Table 5-2 Summary of the risk characterisation calculations for dung organisms using *Scatophaga stercoraria* toxicity data from Rombke *et al*., (2009)

	PEC (µg/kg ww)	**Toxicity (µg/kg ww)**	**Assessment factor**	**PNEC (µg/kg ww)**	**RQ (PEC/PNEC ratio)**
Phase II Tier A	5,000 ($PEC_{dung\text{-}initial}$) Assumes 100% excretion over 1 day	Survival EC_{50}:18 (*Scatophaga stercoraria*)	100	0.18	27,778
Phase II Tier A	5,000	Survival NOEC: 9.3 (*Scatophaga stercoraria*)	10	0.93	5,376
Phase II Tier B	180 ($PEC_{dung\text{-}refined}$) Max. measured concentration (Section 3.4)	Survival NOEC: 9.3 (*Scatophaga stercoraria*)	10	0.93	**193.5**

Table 5-2 concerns the risk assessment dung fauna. The initial conservative estimate of ivermectin risk presented in Equation 1-6 can be refined using the most realistic data available i.e. the maximum concentration measured in freshly excreted manure from treated cattle and the lowest observed concentration to affect survival (allowing the use of a smaller assessment factor). The resulting risk quotient (RQ) for dung organisms was 194, this is still significantly greater than 1, indicating an unacceptable risk to dung fauna.

However, this is still a highly simplified assessment and not representative of real exposure in the field. A number of key factors have been ignored, meaning that risks are likely to be greatly over-estimated. Firstly, exposure concentrations are not constant as assumed in the calculations in Table 5-1, but are pulsed, with the timing and duration of residues in the field depending on the treatment times, treatment type (e.g. injection or pour-on) and the number of times the cattle are treated. Secondly, the timing of the application of the parasiticide to the animals may or may not coincide with periods where sensitive life stages of insects are present in the dung. Studies have demonstrated the range of seasonal activity periods when dung is occupied by dung insects (e.g. Floate & Gill, 1998; Lee & Wall, 2006a; Gittings & Giller, 1999; Giller & Doube, 1994). Parasiticide residues in the field must coincide with the activity of the insect life-stage sensitive to the residues (e.g. larval stage) for the parasiticide to have a direct impact on the insect. Other factors to consider are degradation and the availability of manure from untreated cattle. If residues are found to degrade under field conditions within the period of time the pats are attractive to soil and dung organisms, then this should also be taken into account. Furthermore, if there are reservoirs of 'safe' manure (from untreated cattle) at an accessible distance, this might also reduce the impact of the treatment. Finally and perhaps most importantly, the assessments described in the Guidance documents do not consider the longer-term impacts at the population level. While field studies where the emergence of insects from pats containing parasiticides is monitored, such as those conducted by Floate *et al.,* (2002) and Iwasa *et al.,* (2005), can help refinement by providing a community level NOEC, this is still an impact measured at one point in time. An insect's life-history characteristics, such as

fecundity and development rates which influence its growth potential, are likely to affect the degree of response to parasiticide exposure.

Population modelling is one method of extrapolating the results of laboratory toxicity testing to the field scale, using the information available to the applicant. The matrix population modelling performed in this study used data on ivermectin based on its measured excretion profile in cattle and recommended dosing strategies, along with insect ecological characteristics such as: fecundity, development rates and survival rates at different densities. The insect data were gathered from the literature and the results of a laboratory toxicity test. The model was used to assess the impact on insect abundance over one year. The results indicated, when density dependence is incorporated into the model, that, despite its high inherent toxicity, ivermectin use in the UK environment results in minimal impact on fast breeding flies such as *Scatophaga stercoraria*. However, the model as it is described here has limitations. Useful indicators of population level effects might include the ability of the population to recover (e.g. time to recovery, probability of extinction). Density dependent models are often used for determining such indicators. Although a density dependent model has been developed here, no satisfactory method of examining the sensitivity of the model to changes in the model parameters has been established since the matrix modelling technique presented can not make use of the most commonly used output metrics for matrix modelling (because the matrix varies over time). Confident conclusions about population level impacts of parasiticide use can not be drawn until these issues have been addressed. In addition, it is important to recognise that as the toxicity data used in the model were from a laboratory-based study using dung spiked with ivermectin, the current model did not consider the potential effects of the two identified metabolites so actual impacts on the population may be higher than predicted. The results from the modelling are however supported by comparable results of a field monitoring study reported by Webb *et al.,* (2007) where the abundance of *S. stercoraria* between April and July over a two year period was assessed and no difference was observed in abundance between pastures of avermectin-treated livestock and those with untreated livestock.

While the modelling was undertaken on *S. stercoraria*, it could easily be extended to model the impact of ivermectin use on other dung insects with very different ecologies, such as multivoltine and univoltine beetles. Indeed, to fully characterise the impact of ivermectin use, an assessment of its environmental safety should be undertaken which accounts for the ecologically- and taxonomically-diverse dung fauna community (Lee & Wall, 2006a; Floate, 1998) rather than just one species (1991).

The results obtained from the studies described in this thesis have raised a number of other questions regarding the risk assessment of ivermectin use in the pasture. This study demonstrated that ivermectin residues can be transported to the soil environment following the degradation of pats in the field, probably as a result of the incorporation of the pat into the soil by the activity of earthworms. A comparison of the maximum measured concentration in soils with data on *F. fimetaria* resulted in risk quotient slightly above one (2.07). However, this maximum concentration was limited to the very top layer of soil. In addition, while only soil beneath the pats was sampled, it is likely that ivermectin residues will only occur in patches immediately below the position of the pats. When considering both the isolated nature of the hotspots and the risk quotient near one, it is likely that soil organisms will not be continuously exposed to high levels - indicating a low risk. However, as degradation tests indicate very slow degradation of ivermectin residues under laboratory conditions the potential for a build-up of residues in the pasture exists. An assessment of ivermectin persistence in soil under environmental conditions and its spatial variation in the pasture would help us understand the risks posed to soil fauna by parasiticide use in the pasture.

5.2 Fate of Ivermectin in the Aquatic Environment

While the main focus of this thesis has been on terrestrial systems, the fate of ivermectin in large-scale aquatic mesocosm studies was also analysed. Ivermectin was found to rapidly dissipate in the water (with a DT_{50} of approximately 4 days) largely by partitioning into the pond sediment. Residues in the sediment were found to be highly persistent, with no degradation occurring

within the 265 days of the study (Sanderson *et al*., 2007). These results indicate that where ivermectin residues do reach aquatic water bodies, they are likely to persist for long periods of time and if there is repeated exposure, residues may accumulate in the sediment. This prolonged exposure may pose a risk to sediment organisms.

5.3 Implications for Environmental Risk Assessment of Parasiticides

The findings of this study have highlighted a number of areas that require consideration during the risk assessment process for parasiticides, these are discussed below.

5.4 Analytical Considerations

A robust extraction and analytical method was developed to quantify ivermectin in manure and soil which was tested and validated under laboratory conditions. However, these methods did in fact yield misleading results when applied to manure samples exposed to field conditions. Initial analysis of manure samples from the field study indicated significant ivermectin degradation within 19 days. These findings would have been in agreement with several other studies reporting rapid degradation of avermectins under field conditions, which have reported DT_{50} ranging from less than 6 days to 30 days for abamectin, doramectin and ivermectin in sheep and cattle manure (Erzen *et al.*, 2005; Halley *et al.*, 1989a; Lumaret *et al.*, 1993). Further investigation, which was only triggered by the 're-appearance' of ivermectin in a later sampling event, demonstrated that the extraction efficiency of the method was strongly affected by the sample moisture content. When the method was refined to address this issue (by re-hydrating samples prior to extraction) and the field study samples re-analysed, ivermectin was found not to degrade within the 38 days of this study. These results highlight the need to carefully evaluate analytical methods, in particular taking account of the changes samples may undergo in response to variable environmental conditions, which may affect the analyte extractability. While this work has highlighted the effect of differences in moisture content, it is

possible that other factors could also be important, such as differences in the physical and chemical properties of the different soil types, aging and other climatic factors.

Effects of environmental variables on extractability have been observed previously by others. For example, when using a solvent based pressurized liquid extraction method, O'Connor *et al.,* (2007) found the extraction efficiency of a range of tetracyclines to vary widely between different soil types, from 22 to 99%, especially with respect to differing clay and organic matter content. Other studies found that a cycle of repeated freeze-thawing action significantly influenced the extractability of pyrene in soils (Zhao *et al.*, 2009). The same study also demonstrated that, for soils which did not undergo freeze-thaw action, an increase in soil moisture content increased the extractability of pyrene in soils aged for 1 year (Zhao *et al.*, 2009). The aging of persistent compound residues in soil has been shown to reduce their extractability and bioavailability to soil organisms (e.g. Northcott & Jones, 2001; Song *et al.*, 2006; Harmsen, 2007). However, further caution should be exercised if the processes of restricting extraction efficiency and bioavailability can be reversed, as shown in this study with the effect of varying moisture content.

Analytical methods therefore need to be validated for the specific situation or scenario in which they will be used, e.g. the same soil type as the study soil, manure from the same animal types, fed on the same diet, and changing effects of exposure taken into account, e.g. aging, or moisture content.

5.4.1 Terrestrial Fate Studies

The potential existence and effects of metabolites need to be considered when undertaking terrestrial fate studies. Recommended methods for testing the biodegradability of veterinary chemicals in the laboratory are currently under development by the CVMP (2009). In these tests, a sample of manure is usually spiked with the compound in question, rather than using manure collected from treated animals and therefore ignores potential metabolites that may be excreted along with the parent compound. Furthermore, no consideration is given to the

dung-soil interface, or the effect of the compound's mobility or soil organisms' activity. Semi-field tests systems such as the Terrestrial Model Ecosystem (TME), an intact soil core naturally seeded with endemic soil organisms (Knacker *et al.*, 2004), or the multispecies soil system (MS3) (e.g. Boleas *et al.*, 2005), in which the soil organisms are added to sieved soil cores, go some way to addressing these issues as they have the potential to integrate fate and toxicity studies on soil organisms. However, the more mobile, and possibly more important organisms, such as dung flies and beetles are currently excluded from the available study systems.

The approach described in this thesis represents a field-based method, more representative of the real-world scenario, using manure from treated animals exposed to the natural succession of dung and soil fauna. A dung fauna community level effect (e.g. NOEC) may be determined, alongside fate studies which can investigate persistence in dung, and provide an opportunity to understand the transport of the compound from dung to the soil compartment.

5.4.2 Assessment of Impact on Terrestrial Fauna

There are a number of possible approaches for the extrapolation of results determined in simple laboratory toxicity tests to help understand the impacts in the real world, such as: species sensitivity distribution (SSDs), field toxicity studies and monitoring studies. The aim of methods such as SSDs and field-based toxicity tests (like the one described above) is to ultimately derive a community level NOEC or EC_{10}. SSDs use the results of controlled laboratory toxicity tests on a number of species to provide an effect concentration that usually protects 95% of the species of interest in a particular system, while a field-based toxicity test can provide an effect concentration for the local dung and soil fauna community. However, neither of these approaches considers the long-term impact or the potential for the dung (and soil) fauna populations to recover from parasiticide induced losses. This aspect can be addressed by field studies monitoring insect abundance (e.g. Webb *et al.*, 2007), but these methods can be expensive and logistically difficult, in particular considering the slow population cycles of univoltine dung beetles.

Population modelling has been demonstrated to be a useful tool in environmental risk assessment. Predictions of population level impacts can be produced based upon information available to industry (e.g. compound excretion rates, recommended use practices) and information on insect ecology available in the published literature. Once the model is parameterised, the method is easy and quick to use. However, some aspects of applying population modelling need further consideration. How best to use modelling results in the environmental risk assessment is one such aspect, an area where regulatory guidance is lacking. One approach could be to consider the time required for a population to recover from the parasiticide treatment, an approach used in the risk assessment of pesticides in the UK. A recent UK workshop on assessing the risks of pesticides to non-target arthropods, advised the previously undefined 'recovery period' to mean recovery in one season, stating that recovery or, the potential for recovery, should be assessed on a case-by-case basis, a method that would also be relevant in the assessment of parasiticides in pastured animals (Environmental Panel of the Advisory Committee on Pesticides, 2002).

The application of population modelling to environmental risk assessment is still an emerging area in which further guidance would be beneficial. One of the objectives of the SETAC advisory group MeMorisk (Mechanistic effect models for ecological risk assessment of chemicals) is to be actively involved in the development of guidance documents on the use of modelling in ecological risk assessment.

The population modelling approach proposed in this thesis could be further developed to improve our understanding of how parasiticides affect dung fauna ecology. The inclusion of density-dependence in the population model is very important as it allows the possibility for the modelled species to recover post-treatment, and for the use of 'time to recover' as a model endpoint. Further study is desirable to better model the density-dependent processes occurring in the field for different dung fauna species.

Another key future development of the model would be to model the effect of parasiticide use on species representing the wide range of ecologies of dung fauna species. The model requires detailed information on the life-history characteristics of the modelled insect, which is difficult to find for individual species, a point previously stressed by Wardhaugh *et al.*, (1998). One possible solution is to develop a series of theoretical dung fauna species, incorporating the life-history characteristics of similar species to cover the range represented in the dung fauna community. The worth of this approach has been demonstrated in the studies by Wardhaugh *et al.*, (1998) and Vale and Grant (2002).

5.4.3 Suggested Methodology for the Assessment of the Risks Posed to Dung Fauna by Parasiticides

Figure 5-1 illustrates how the approaches described above may be employed in the risk assessment process. The first refinement of the PEC/PNEC calculation is performed using the toxicity data and the measured concentrations reported in the ADME studies. Should an unacceptable risk be determined at this stage then the subsequent route should be selected on a case-by-case basis. The results from the population modelling may inform the design of field tests and monitoring studies, while the results of the fate investigations undertaken in the field may (if pertinent) be incorporated into the population modelling.

5.5 Recommendations for Future Work

One major output of this thesis is the development of a modelling-based approach for estimating the effects of parasiticides on dung organisms. The approach has been illustrated using one compound and one fly species. We believe that the approach provides a useful addition to the tool box for use in the risk assessment of veterinary medicines. However further work is required before the approach can be applied routinely. Future work aimed at further refining the risk assessment for veterinary medicines, and parasiticides in particular, in the terrestrial environment should focus on the following:

- Appropriate development and validation of the analytical methods used to quantify compound residues in the field. This study has demonstrated that methods found to be robust in the laboratory may not be appropriate when applied to other situations, such as under field conditions where changes in climate may influence the characteristics of the matrix. We need to develop a better understanding of how environmental conditions can alter analytical performance and formulate systematic method development strategies (e.g. recommendations on selection of validation matrices and suites of solvents to use in the extraction process) that address appropriate variations in conditions.

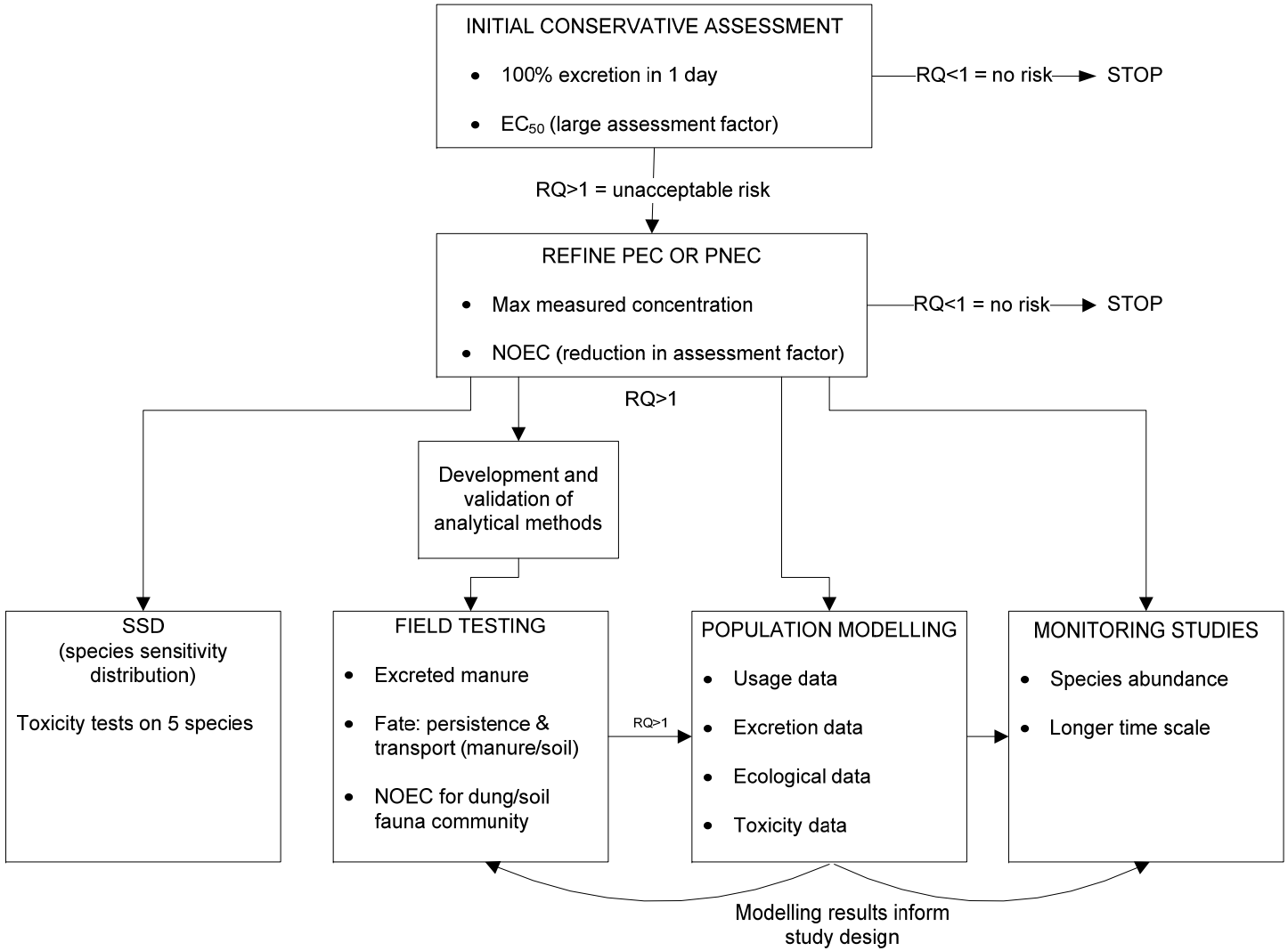


Figure 5-1 Procedure for assessing risk to dung fauna

- The formation of non-extractable residues in soil is known to reduce the compounds toxicity and bioavailability (Barriuso *et al.*, 2008). However a change in the matrix, such as an increase in the moisture content, may cause residues to become more easily extracted. This would suggest that, in these circumstances, non-bioavailable residues may become available again and once again pose a risk to exposed biota. Further investigation is required to better understand the factors controlling the non-extractability of VMP residues and their re-release following a change in environmental conditions.

- This study has demonstrated that highly-adsorptive compounds such as ivermectin can in fact be transferred to the soil compartment. To fully understand the risks posed to soil fauna by a parasiticide used in pastured livestock, an assessment of its persistence in soil needs to be undertaken. In addition, as residues are most likely to be transferred to the soil directly beneath pats, concentrations in soil are likely to be highly heterogeneous in space. The methods set out in the guidance documents available (VICH, 2004; CVMP, 2008) assumes a uniform application. Further investigation is required to understand the implications for these patches of contaminated soil and how they will affect soil fauna.

- To appropriately assess the impact of parasiticide use on the dung fauna community as a whole, the population model developed in this thesis should be extended to cover a range of dung fauna species, perhaps using a theoretical species approach where the model is parameterised for a set of hypothetical species designed to cover the breadth of species traits that would likely be observed in a typical pasture environment in different regions.

- The modelling approach could be further improved by incorporating a spatial aspect, enabling consideration of the 'dilution' effect where dung fauna have access to reservoirs of manure from untreated cattle. In addition, confidence intervals may be applied to the model endpoints

(such as time to recovery) if the appropriate model parameters are made stochastic. The sensitivity analysis of the density-independent model highlighted the importance of insect development rates, and since these development rates are key factors in determining the population growth rate they will influence the rate at which populations recover from parasiticide-induced mortality. The sensitivity of the model to these parameters indicates that these parameters may be ideal candidates for introducing stochasticity. However, the matrix models may not be the best approach for exploring stochasticity. Other population modelling methods, such as individual based modelling (IBM), could be explored since they may be better suited to introducing stochasticity and spatial aspects.

- A better understanding of parasiticide usage patterns and livestock farming practices would help in the development of a range of usage scenarios, such as those employed by Sherratt *et al.,* (1998), where scenarios were developed for different regions: Northern Europe, Southern Europe, South Africa and Australia. The model scenarios could include differences in: the number of times the parasiticide is applied, the proportion of the cattle treated, when cattle are turned out to pasture (or pastured all year), and model the impacts on local dung fauna, with phenologies observed in those regions.

- The mechanisms for incorporating the effect of carrying capacity within density dependent models differ between species. A better understanding of these mechanisms and parameterisation under more natural conditions would further improve the density-dependent model.

- Perhaps one of the most important recommendations for further work is to determine how best to use these models in risk management decisions. In addition, instead of an either-or approach, the results of population modelling could be used to inform the design of field tests and

monitoring studies, directing efforts to those species suspected to be the most vulnerable to parasiticide use.

- We should consider the evaluation and calibration of these models. By understanding the relationships between field observations and model output it may be possible to develop guidance on how to interpret the model predictions.

- Several studies into the effect of parasiticides on dung fauna have demonstrated non-lethal effects such as changes to development rates and fecundity. The matrix model described here is ideally suited to incorporate these effects.

- Finally, although perhaps difficult to do, the indirect effects of parasiticide use should be assessed. Several species in the pasture food chain are known to predate on dung fauna, including increasingly rare species of bats, birds and insects. A complete assessment of the risks posed by parasiticides to terrestrial fauna may need to take a holistic approach, considering the impact on soil and dung fauna and those organisms which predate them.

Appendix A – Model Code in MatLab

A.1 Model.m – main file

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Model5
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Includes initial values for variables, user-entered parameters, initialises
% the matrix, calls the iteration of the model and reports results in a
% graph and a table to be copied into excel.

%%%%%GLOBAL VARIABLES at the start %%%%%%%%
global ControlPopulationMatrix;
global TreatedPopulationMatrix;
global Leslie;
global FieldConc;
global EggTime;
global ImmatureTime;
global BrewTime;
global MaxLifespan;
global ClutchSize;
global SurvivalEggtoImmature;
global DensitySlope;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% OUTSIDE QUESTIONS – Not used in final version
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Ask questions for which answers stay the same across runs outside
% the big loop

% Duration = variable
% input ('string') a function that prompts the user for input from the
% keyboard, displaying the 'string'

%Duration = input('How many days will the model run? ');
Duration = 248;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% TREATMENTS AND TOXICICITY
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Set up the field concentrations of the drug on each day of the study
% Empty field concentration vector
FieldConc = zeros(1, Duration);

% Ask the user how many treatments to administer – not used in final version
%NumberOfTreatments = input('How many treatments? ');
```

```
NumberOfTreatments = 3;


% Ask the user to select t (for topical) or i (for injection) – not used in final
% version
% 's' at the end means I'm expecting text input
% Formulation = input('select t (for topical) or i (for injection) ', 's');

Formulation = 'i';

% load the relevant excretion file for the treatment type
if Formulation == 't'
   ExcretionProfile = dlmread('topical.txt', '\t');

elseif Formulation == 'i'
   ExcretionProfile = dlmread('25_excretion_34d.txt', '\t');

else
   'Formulation not recognised'
end

% Load the excretion profile into the field concentrations – done automatically in
% the final version
%for TreatmentNumber = 1:1:NumberOfTreatments
% DayOfTreatment = input('Day of treatment? ');
% Fieldcon (= Row 1, Columns dayoftreatment to dayoftreatment
%  + durationoftreatment) = excretionprofile
%  FieldConc(1, DayOfTreatment:(DayOfTreatment+size(ExcretionProfile, 2)-
%   1)) = ExcretionProfile(1,1:end);
%end

FieldConc(1, 86:(86+size(ExcretionProfile, 2)-1)) = ExcretionProfile(1,1:end);
FieldConc(1, 121:(121+size(ExcretionProfile, 2)-1)) = ExcretionProfile(1,1:end);
FieldConc(1, 156:(156+size(ExcretionProfile, 2)-1)) = ExcretionProfile(1,1:end);

% Link toxicity to day (via concentration), through mortality_calc
% Build this in to leslie matrix via iterate file

LastDays = 50;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% LIFE-HISTORY PARAMETERS – fixed in final version
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Lifespan = input('What is the maximum lifespan in days? ');
% BrewTime = input ('How many days to mature clutch of eggs? ');
% ClutchSize = input ('How many eggs in one clutch? ');
% EggTime = input ('How many days from egg to immature adult? ');
% ImmatureTime = input ('How many days from immature adult to egg-layer? ');

MaxLifespan = 80;
```

```
BrewTime = 7;
ClutchSize = 25;
EggTime = 33;
ImmatureTime =14;
SurvivalEggtoImmature = 0.82;
DensitySlope = -3.61E-07;
SurvivalImmaturetoLifespan = 0.389;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% SETTING UP LESLIE MATRIX
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Create a Leslie matrix which show how the animals move from one lifestage
% to the next - first create a matrix of zeros
Leslie = zeros(MaxLifespan, MaxLifespan);

% Aging - Increase the ages of everything by moving them to the next stage
% (a day older)

% Then change all the survival values (P) to 1 followed by changing the
% boundary values (eggs to immatuures and survival of egg-layers).
% For loop: repeat everything between for and end with i(row) = 2, i = 3, i = 4,
% ..., i = Lifespan

for i = 2:1:MaxLifespan
   j = i-1;
   Leslie(i, j) = 1;
end

% i = 2, and loop is repeated until i = Lifespan
% From 2, add 1 each time until Lifespan is reached

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% SETTING SURVIVAL AT STAGE BOUNDARIES
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Eggs to Immature Survival: Not all eggs emerge
% EggTime+1 row in Leslie to calculate No. of immatures. EggTime: Column of
% the oldest eggs (now pupae)  (sets one element to 0.82)

% = Proportion of the oldest eggs that become immatures is set in iterate

% Immature to Lifespan
for i = EggTime+ImmatureTime+1:BrewTime:MaxLifespan
   j = i-1;
   Leslie(i, j) = SurvivalImmaturetoLifespan;
end

%Egg-laying
for j = (EggTime + ImmatureTime + 1):BrewTime:MaxLifespan
   Leslie(1, j) = ClutchSize;
```

```
end


%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% CREATING POPULATION MATRICES (1 CONTROL, 1 TREATED)
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Intialise the matrix of the population. 1st dimension is life stages,
% second is time.

% Set the current day to 1
CurrentDay = 1;

% Create a matrix of zeros for the population in each class on each day.
ControlPopulationMatrix = zeros(MaxLifespan, Duration);
TreatedPopulationMatrix = zeros(MaxLifespan, Duration);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% SEEDING POPULATION MATRIX
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Seeding population with a normal distribution of emergence
Emergence = dlmread('em_div200.txt', '\t');

ControlPopulationMatrix(1:size(Emergence, 1),1) = Emergence (1:end, 1);
TreatedPopulationMatrix(1:size(Emergence, 1),1) = Emergence (1:end, 1);

% The iterate function creates the next day's population
while CurrentDay < Duration
  CurrentDay = iterate(CurrentDay);
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% OUTPUT
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% PERCENTAGE LOSS OVER SET NO. DAYS (LAST DAYS) –
% ORIGINAL METHOD
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% = ((median treated100d - median control100d)/ median control100d) * 100
ControlAdults = sum(ControlPopulationMatrix(EggTime+1: end , :));
TreatedAdults = sum(TreatedPopulationMatrix(EggTime+1: end , :));

ControlAdultsTransposed = ControlAdults';
TreatedAdultsTransposed = TreatedAdults';

%Adult population in LastDays, makes 2 temporary vectors
ControlLastDays = ControlAdults(Duration-LastDays+1:end);
TreatedLastDays = TreatedAdults(Duration-LastDays+1:end);

ControlLastDays = ControlLastDays';
```

```
TreatedLastDays = TreatedLastDays';


%Calc median value
MedianControlLastDays = median(ControlLastDays);
MedianTreatedLastDays = median(TreatedLastDays);

%Calc mean value
MeanControlLastDays = mean(ControlLastDays);
MeanTreatedLastDays = mean(TreatedLastDays);

%Calc percentage loss and store in a table
PercentageLoss = ((MeanControlLastDays  -
     MeanTreatedLastDays)/MeanControlLastDays)*100;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% GRAPHS
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
semilogy(TreatedAdults, 'r');
hold on
semilogy(ControlAdults, 'g');
hold on
legend('Treated','Control');
xlabel('Days');
ylabel('Number of adults');
```

A.2 iterate.m – daily increment function file

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% ITERATE
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Iterate: The function that performs the daily modelling.
% Takes the current day, accesses population in the model for the current day
% adds one to the day, works out the new population and stores back in the
% population matrix
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% The function that performs actions on each day of the model:
function NextDay = iterate(Day);

% Define the global we're using inside the function
% Use the PopulationClassesMatrix defined in Model.m
global ControlPopulationMatrix;
global TreatedPopulationMatrix;

% Use the Leslie matrix defined in Model.m;
global Leslie;
```

```
global EggTime;
global ImmatureTime;
global BrewTime;
global Lifespan;
global ClutchSize;
global SurvivalEggtoImmature;
global DensitySlope

% To begin add one to the day
NextDay = Day + 1;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% TOXICTY and DENSITY DEPENDENCE
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% We want to vary emergence according to concentration

% = Proportion of Day 1 eggs that become 2 days old
Leslie(2, 1) = 1;
% = Proportion of the oldest eggs that become immatures.

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% CONTROL POPULATION – DENSITY DEPENDENT
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Larval density dependence using Amano 1983
% y = -3.61E-07 * No. larvae + 1.09 LarvalDensityFactor = 1.09 + DensitySlope
%        * ControlPopulationMatrix(EggTime,Day);

% cap it to 1 (100% survival if density is too low to have an effect)
%if LarvalDensityFactor > 1
%    LarvalDensityFactor = 1;
%end
%if LarvalDensityFactor < 0
%   LarvalDensityFactor = 0;
%end
%% DENSITY DEPENDENT (CONTROL):
%Leslie(EggTime+1, EggTime) = SurvivalEggtoImmature *
%     LarvalDensityFactor;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%% DENSITY INDEPENDENT(CONTROL):
Leslie(EggTime+1, EggTime) = SurvivalEggtoImmature;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
ControlPopulationMatrix(:, [NextDay]) = Leslie*ControlPopulationMatrix(:,
    [Day]);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% TREATED POPULATION
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% = Proportion of Day 1 eggs that become 2 days old
```

```
Leslie(2, 1) = 1 - mortality_calc(Day);
% = Proportion of the oldest eggs that become immatures.

% Larval density dependence from Amano 1983
% y = -3.61E-07 * No. larvae + 1.09
%LarvalDensityFactor = 1.09 + DensitySlope *
%    TreatedPopulationMatrix(EggTime,Day);

% cap it to 1 (100% survival if density is too low to have an effect)
%if LarvalDensityFactor > 1
%    LarvalDensityFactor = 1;
%end
%if LarvalDensityFactor < 0
%    LarvalDensityFactor = 0;
%end
%% DENSITY DEPENDENT (TREATED):
%Leslie(EggTime+1, EggTime) = SurvivalEggtoImmature *
%      LarvalDensityFactor;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%% DENSITY INDEPENDENT (TREATED):
Leslie(EggTime+1, EggTime) = SurvivalEggtoImmature;
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

TreatedPopulationMatrix(:, [NextDay]) = Leslie*TreatedPopulationMatrix(:,
[Day]);
```

A.3 mortalityCalc.m – function file for calculating the drug effect

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% MORTALITY_CALC
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Linking insect mortality to field day

% Function: Mortality, that calculates egg mortality on a given day
% based on field concentration of the drug

% Function that returns the mortality on a given day
% DrugMortality = the variable that we return

% DrugMortality is the proportion that die due to drug exposure, on top of
% any 'natural' mortality
function DrugMortality = mortality_calc(FieldDay)

% Define the global we're using inside the function
% FieldConc
global FieldConc;
```

```
% to look up the concentration on any given field day:
Conc = FieldConc (1,FieldDay);

% Calculate DrugMortality based on conc in field day
% using formula calculated in R by Mark.
% Need to cap the mortality at 100% (DrugMortality of 1)
% DrugMortality = A*(1-EXP(-C*X))

A = 108.2;
C = 0.004901;

if Conc == 0
   DrugMortality = 0;
else
   DrugMortality = (A*(1-exp(-C*Conc)))/100;

   if DrugMortality >1
      DrugMortality = 1;
   end
end
```

Appendix B - Publications

6 Exposure Assessment of Veterinary Medicines in Terrestrial Systems

Louise Pope, Alistair Boxall, Christian Corsing, Bent Halling-Sørensen, Alex Tait, and Edward Topp

6.1 INTRODUCTION

It is inevitable that during their use, veterinary medicines will be released to the terrestrial environment. For hormones, antibiotics, and other pharmaceutical agents administered either orally or by injection to animals, the major route of entry of the product into the soil environment is probably via excretion following use and the subsequent disposal of contaminated manure onto land (Halling Sorensen et al. 2001; Boxall et al. 2004). Drugs administered to grazing animals or animals reared intensively outdoors may be deposited directly to land or surface water in dung or urine, exposing soil organisms to high local concentrations (Sommer et al. 1992; Halling Sorensen et al. 1998; Montforts 1999).

The fate and subsequent transport of a given medicine in soil will depend on its specific physical and chemical properties, as well as site-specific climate conditions that are rate limiting for biodegradation (e.g., temperature) and soil characteristics (e.g., pH, organic matter, or clay content) that determine availability for transport and for biodegradation. For example, the propensity for sorption to soil organic matter (the K_{oc}) will influence the potential for mobility through leaching. Overall, knowledge of soil physical and chemical properties combined with data from environmental fate studies will confirm if a substance is classified as biodegradable, persistent, or a risk to other compartments (e.g., surface water or groundwater).

In this chapter, we describe those factors and processes determining the inputs and fate of veterinary medicines in the soil environment. Models used for estimating concentrations of veterinary medicines in animal manure and in soil, and the fate and behavior of these medicines once in the terrestrial environment, are also described. We conclude by identifying a number of knowledge gaps that should form the basis for future research.

6.2 ABSORPTION AND EXCRETION BY ANIMALS

Knowledge about the kinetics of the veterinary medicine after application to the target animals is of tremendous relevance within the development of a veterinary medicinal product. This is obtained from the adsorption, distribution, metabolism, and excretion (ADME) study, which is usually undertaken with a radiolabeled parent compound. As indicated in Chapter 2, the degree of adsorption will vary with the method of application, and can range from a few percent to 100%. Once absorbed the active ingredient may undergo metabolism. These reactions may result in glucuronide or sulfate conjugates, or may produce other polar metabolites that are excreted in the urine or feces. The parent compound may also be excreted unchanged, and, consequently, animal feces may contain a mixture of the parent compound and metabolites. A general classification of the degree of metabolism for different types of veterinary medicine is given in Table 6.1. General assumptions may be revised where detailed ADME investigations are available (Halley et al. 1989a). ADME investigations may also provide information on the excretion of a parent compound, the amount and nature of excreted metabolites, and how these vary with application method. Metabolism data will help to identify whether the parent compound is the correct substance for further environmental assessment, or whether a major metabolite, already formed in and excreted by the animal, should be the relevant one for assessment (e.g., pro-drugs).

The formulation of veterinary medicines (e.g., aqueous or nonaqueous), the dosage, and the route of administration are key factors in determining the elimination profile for a substance. Animals tend to be treated by injection (subcutaneously or by intramuscular injection), via the feed or water, topically (as a pour-on, spot-on, or sheep dip application), by oral drench, or via a bolus releasing the drug

TABLE 6.1
General trend for the degree of metabolism of major therapeutic classes of veterinary medicines

Therapeutic class	Chemical group	Metabolism
Antimicrobials	Tetracyclines	Minimal
	Potentiated sulphonamides	High
	Macrolides	Minimal
	Aminoglycosides	Minimal–high
	Lincosamides	Moderate
	Fluoroquinolones	Minimal–high
Endoparasiticides—wormers	Azoles	Moderate
Endoparasiticides—wormers	Macrolide endectins	Minimal–moderate
Endoparasiticides—antiprotozoals	—	Minimal–high
Endectocides	Macrocyclic lactones	Minimal–high

Note: Classification: minimal (< 20%), moderate (20–80%), high (> 80%).
Source: Classification taken from Boxall et al. (2004).

TABLE 6.2
Parasiticide formulations available in the United Kingdom

Parasiticide	Cattle	Sheep
Albendazole	Oral	Oral
Cypermethrin	—	Dip
Deltamethrin	Pour-on	
Spot-on	Spot-on	
Diazinon	—	Dip
Doramectin	Subcutaneous injection	Intramuscular injection
Eprinomectin	Pour-on	—
Fenbendazole	Oral suspension	
Oral bolus		
Feed	Oral suspension	
Ivermectin	Injection	
Pour-on	Injection	
Oral		
Levamisole	Oral	
Pour-on	Oral	
Morantel	Bolus	—
Moxidectin	Injectable	
Pour-on	Injectable	
Oral drench		
Oxfendazole	Pulse release bolus	
Oral	Oral	
Triclabendazole	—	Oral

Source: National Office of Animal Health (2007).

over a period of time. Many medicines commonly used are available in 1 or more application types and formulations (e.g., Table 6.2). For example, fenbendazole is available in the United Kingdom as an oral drench for cattle and sheep at different concentrations and as a bolus for cattle, continuously releasing fenbendazole for 140 days.

Pour-on treatments result in higher and more variable concentrations than injectable treatments, and compounds are excreted more rapidly following oral applications. Most studies on this in the literature concern the different methods of administering ivermectin. (Herd et al., 1996) investigated the effect of 3 ivermectin application methods upon residue levels excreted in cattle dung over time (Figure 6.1). Ivermectin residues following a pour-on application resulted in a higher initial peak of 17.1 mg kg^{-1} (dry weight) occurring 2 days after treatment. Comparable results were obtained by (Sommer & Steffansen, 1993), where peak excretion of 9 mg kg mg kg^{-1} (dry weight) occurred 1 day after pour-on. Subcutaneous injection was found to result in a slightly later and considerably lower peak excretion of 1.38 mg kg^{-1} (dry weight) after 3 days by Herd et al. (1996). Sommer

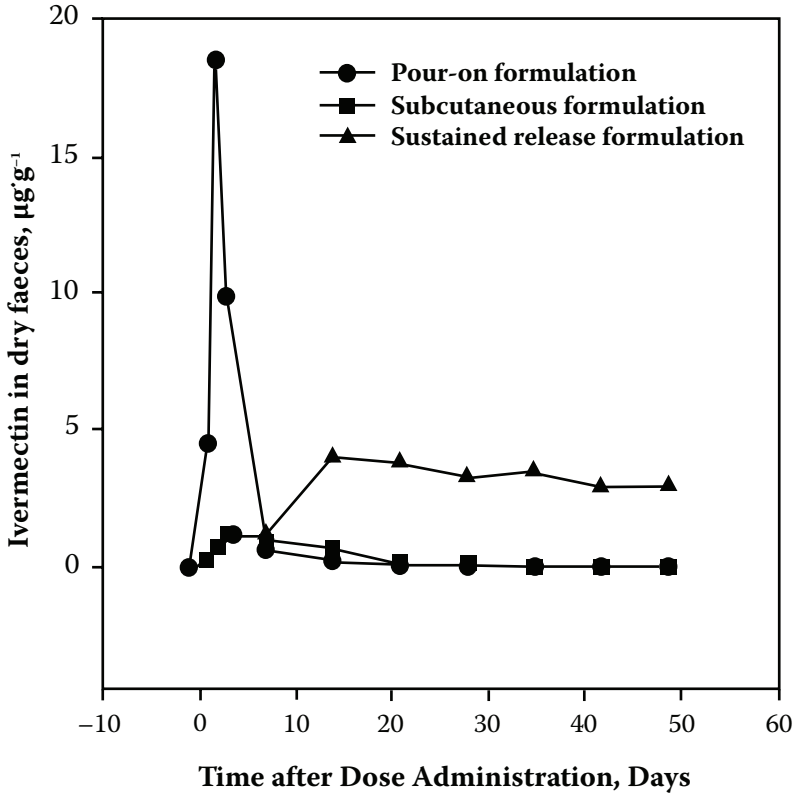


FIGURE 6.1 Excretion profiles of ivermectin following 3 different application methods. *Source*: Reprinted with permission from Herd et al. (1996).

and Steffansen (1993) reported a peak of 3.9 mg kg^{-1} (dry weight) after 2 days. After approximately 5 days, both studies found that both pour-on and injection residue levels declined at a similar rate. Sommer et al. (1992) provide an example of how the considerations above can affect exposure for ivermectin applied to cattle by subcutaneous or topical (pour-on) application. Maximum excretion concentration (C_{max}) may differ by at least a factor of 2. In Sommer et al.'s (1992) data, values of 4.4 ppm versus 9.6 ppm were obtained. The value for t_{max} may also be slightly different due to absorption and distribution processes, whereas the overall time of excretion of relevant amounts may be similar.

Differences in peak excretion levels between pour-on and injectable ivermectin formulations (e.g., Figure 6.1) were attributed to a slower release from the subcutaneous depot, rapid absorbance through the skin, and differences in the dose rate (Herd et al. 1996). However, (Laffont et al., 2003) found the major route of ivermectin absorbance after pour-on to be oral ingestion after licking, and not absorbance through the skin (accounting for 58% to 87% and 10% of the applied dose, respectively). This led to high variability (between and within animals) in

fecal excretion, and, in addition, most of the applied dose was transmitted directly to the feces. Doramectin and moxidectin were also found to be transferred via licking to untreated cattle ((Bousquet-Melou et al., 2004). It would therefore appear that fecal residues of veterinary medicines following pour-on application are more difficult to predict than is the case for other forms of application.

Several studies have indicated that residues are excreted more rapidly following oral (aqueous) treatment compared to injectable (nonaqueous) treatments. When comparing both treatments to sheep, (Borgsteede, 1993) demonstrated that the injectable formulation of ivermectin had a longer resident time in sheep than the oral formulation. (Wardhaugh & Mahon, 1998) found that dung from cattle treated with injectable ivermectin remained toxic to dung containing dung-breeding fauna for a longer period of time compared to dung from orally treated cattle. As the two treatments were of the same dose, it was concluded that the oral formulation is eliminated more rapidly than the injectable formulation. The pattern of excretion following treatment using a bolus is clearly very different. Boluses are designed to release veterinary medicines over a prolonged period of time, as either a pulsed or sustained release. Following use of the sustained-release bolus, Herd et al. (1996) found that fecal ivermectin levels remained relatively constant at a mean of 0.4 to 0.5 mg kg^{-1} (dry weight) from approximately 14 days after application to the end of the study.

After application the active ingredient may be excreted as the parent compound and/or metabolites in the feces or urine of the animal. Figure 6.2 shows

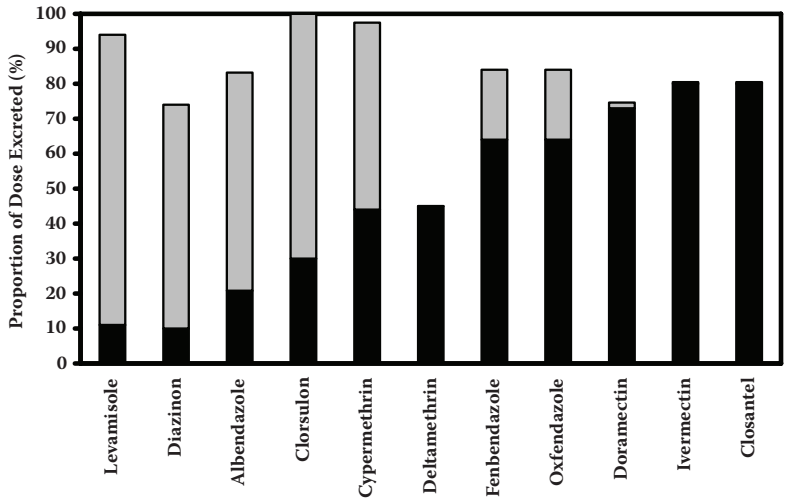


FIGURE 6.2 The percentage of the applied dose excreted in the dung (in black) and urine (in gray), as parent molecule and/or metabolites. *Source*: Inchem (1993), European Agency for the Evaluation of Medicinal Products (1999), Inchem (2006), (Hennessy et al., 2000; Hennessy et al., 1993b; Paulson & Feil, 1996; Hennessy et al., 1993a; Juliet et al., 2001; Croucher et al., 1985).

the proportion of the applied dose excreted in the dung or urine for a range of parasiticides used in the United Kingdom for pasture animals. The avermectins as a group (e.g., ivermectin and doramectin) tend to be excreted in the feces, with only a small proportion of the applied dose detected in the urine (Chiu et al. 1990; (Hennessy et al., 2000). However, there appears to be a large variation in the excretion route of the benzimidazoles, with the applied dose of albendazole and oxfendazole largely excreted in the urine and feces, respectively (Hennessy et al., 1993b; Hennessy et al., 1993a).

Veterinary medicines excreted in urine tend to be extensively metabolized. For example, when animals are treated orally with levamisole a large proportion of the applied dose is detected in the urine, whereas the parent molecule is not (Paulson & Feil, 1996). Diazinon is also readily metabolized, with 73% to 81% of the applied dose excreted in the urine, and less than 1% present as diazinon (Inchem 1970). Veterinary medicines excreted via feces tend to contain large proportions of the unchanged parent molecule. For example, a large proportion of applied radiolabeled ivermectin (39% to 45%) was excreted in feces as the parent compound ((Halley B. A., 1989). In addition, 86% of the fecal residues of eprinomectin (closely related to ivermectin) were parent compound (Inchem 1998). Closantel is also poorly metabolized, with 80% to 90% of the fecal residues excreted as unchanged closantel (Inchem 1996).

Residue data in target (food-producing) animals used to define withdrawal periods may also be used to give an indication of the potential for bioaccumulation in the environment. However, it must be noted that the compound under consideration should be the same as that for which the withdrawal data are generated, and also be of relevance in the environment. Long withdrawal periods of several weeks may indicate such a potential for accumulation.

6.3 FATE DURING MANURE STORAGE

For housed animals, the veterinary medicine will be excreted in the feces or urine, and these will then be collected and stored prior to use as a fertilizer. During the storage period, it is possible that the veterinary medicines will be degraded. No validated or standardized method for assessing the fate of veterinary medicines in manure at either the laboratory or field level exists, and tests in existing pesticide or OECD guidelines do not cover these aspects. In many confined animal and poultry production systems, waste is stored for some time, during which a transformation of veterinary medicines could occur prior to release of material into the broader environment. Various production systems typically store waste as a slurry; others store it as a solid (Table 6.3). Factors that control dissipation rates and pathways such as temperature, redox conditions, organic matter content, and pH will vary widely according to the storage method employed and climatic conditions. Manure-handling practices that could accelerate veterinary medicine dissipation (e.g., composting) offer an opportunity to reduce environmental exposure significantly.

TABLE 6.3
Commonly employed practices for manure storage and handling

System	Manure stored as	Treatment options[a]
Poultry broiler	Solid (mixing with bedding)	Composting
Poultry layer	Slurry	Static storage, aeration
Beef	Solid	Composting
Dairy	Slurry	Static storage, anaerobic digestion
Swine	Slurry	Static storage, aeration, composting, anaerobic digestion

[a] Fecal material will typically be mixed with some bulking agent (e.g., straw or sawdust) prior to composting. Stored slurry can be aerated by pumped-in air or passively with wind-driven turbines (e.g., Pondmill). Both aerobic composting and anaerobic digestion (for biogas production) will result in increased temperature.

The choice of matrix should depend upon the proposed use of the compound (e.g., cattle, pig, or poultry). The matrix is less likely to influence the degradation pathway than the conditions (aerobic or anaerobic); therefore, an aerobic study in cattle manure is an acceptable surrogate for an aerobic study in pig or poultry litter, although the moisture content could be an influencing factor for some compounds.

It is important to consider the measured concentrations of veterinary medicines in the manure, manure type, storage conditions in the tank, mode of medication, agricultural practice, solids concentration, organic carbon concentration, water content, pH, temperature, and redox conditions in different layers of the tank, as all these factors can influence the degradation process. Degradation may also be influenced under methanogenic, denitrifying, and aerobic conditions. The deconjugation rate of excreted veterinary medicines in manure may be significant and require further study under the relevant conditions.

Laboratory degradation studies of active substances in soil may not be sufficient to predict degradation rates in dung/manure (Erzen et al. 2005). Data are available on the persistence in manure of a range of commonly used classes of antibiotic veterinary medicines (reviewed in Boxall et al. 2004). Sulfonamides, aminoglycosides, beta-lactams, and macrolides have half-lives of 30 days or lower, and are therefore likely to be significantly degraded during manure/slurry storage (although no data are available on the fate of the degradation products). In contrast, the macrolide endectin, ivermectin, tetracyclines, and quinolones have longer half-lives and are therefore likely to be more persistent. Results giving degradation rate coefficients of the different veterinary medicines in manure are not necessarily related to agricultural practice when handling manure, although degradation rates in manure are generally faster than those in soil. For example, under methanogenic conditions the degradation half-life for tylosin A was less than 2 days (Loke et al. 2000). We recommend that systematic experimental

determination of veterinary medicine persistence in appropriate manures incubated under realistic conditions should be performed.

6.4 RELEASES TO THE ENVIRONMENT

For housed animals, the main route of release of veterinary medicines to the soil environment will be via the application of manure or slurry to soils as a fertilizer. In most jurisdictions, regulations and guidelines that mandate manure application practices are based on crop nitrogen or phosphorus needs and site-specific considerations, including climate and land characteristics. Manure application rates, manure application timing, manure incorporation into soil, suitable slope, and setback (buffer) distances from surface water may be specified or required. These best management practices (BMPs) are designed to protect adjacent water resources from contamination with enteric bacteria or nutrients. It remains to be determined if these practices are suitably protective of exposure from veterinary medicines. The characteristics of these practices are summarized in Table 6.4.

Although inputs from housed, intensively reared animal facilities tend to be considered the worst case in terms of environmental exposure, in some instances the pasture situation may be of more concern, particularly when considering potential effects on dung fauna. Compounds in manure stored prior to application to the land will have the opportunity to undergo anaerobic degradation, whereas

TABLE 6.4
Characteristics of manure type or application of best management practices (BMP) that can influence the persistence of veterinary medicines in soil

Factor	Features influencing persistence
	Manure type
Solid	Heterogeneity of application and poor soil contact, diffusivity of oxygen
Slurry	Immediate contact with soil, moisture available for microbial activity, risk of off-site movement
Chicken litter	Heterogeneity of application, high proportion of cellulolytic material (straw, wood shavings, sawdust)
	Application method
Broadcast (surface application)	Poor contact with soil, dessication, exposure to sunlight, risk of off-site movement
Broadcast (incorporated)	Good contact with soil, lower risk of off-site movement
Injection	Good contact with soil, lower risk of off-site movement
	Cropping
Standing crop	Rhizosphere stimulation of biodegradation
Bare soil	Evapotranspiration moisture reduction

veterinary medicines given to grazing animals will usually be excreted directly to the land.

The presence of parasiticide residues in the pasture environment will depend on a number of factors including method of medicine application, degree of metabolism, route of excretion (via urine or feces), and persistence in the field. In addition, at the larger scale, factors such as treatment regime, stocking density, and proportion of animals treated will also influence concentrations in the field. The following sections discuss the factors that influence the likely concentration of parasiticide residues.

6.5 FACTORS AFFECTING DISSIPATION IN THE FARM ENVIRONMENT

"Dissipation" as originally defined for pesticides is the decrease in extractable pesticide concentration due to transformation (both biological and chemical) and the formation of nonextractable or "bound" residues with the soil (Calderbank 1989). The same definition is used here for veterinary medicines. In the following sections, we describe those factors and processes affecting dissipation in dung and soil systems.

6.5.1 Dissipation and Transport in Dung Systems

For pasture animals, once excreted, veterinary medicines and their metabolites may break down or persist in the dung on the pasture. Drug residues in dung may be subject to biodegradation, leaching into the soil, or photodegradation, or be physically incorporated into the soil by soil organisms. Persistence of residues in the field will be heavily influenced by climatic conditions. Differences in location and season will affect both chemical degradation and dung degradation. Results from studies of avermectin persistence in the field ranged from no degradation at the end of a 180-day study in Argentina to complete degradation after 6 days (Lumaret et al., 1993; Suarez et al., 2003). In laboratory studies there is also enormous variation in the degradation rate with soil type and the presence or absence of manure (Bull et al. 1984; Halley et al. 1989a; (Halley et al., 1989; Lumaret et al., 1993; Sommer & Steffansen, 1993; Suarez et al., 2003; Erzen et al., 2005; Bull et al., 1984). (Mckellar et al., 1993) reported consistently lower morantel concentrations in the crust of cow pats compared to the core over 100 days, suggesting that surface residues were subject to photolysis. However, as there is little exposure to sunlight within the dung pat, this was judged unlikely to present a significant route of degradation overall.

At the field scale, the residence time in the field and the overall concentration of veterinary medicines in dung will be affected by a number of factors, including frequency of treatments in a season, stocking density, and the proportion of animals treated. Pasture animals may be treated with veterinary medicines at different times during the grazing season and at different frequencies. For example, the recommended dosing for cattle using doramectin in Dectomax injectable

formulation is once at turnout (around May in the United Kingdom) and again 8 weeks later (National Office of Animal Health [NOAH] 2007). Ivomec classic, a pour-on containing ivermectin, recommends treating calves 3, 8, and 13 weeks after the first day of turnout (NOAH 2007). However, the moxidectin treatment used in Cydectin pour-on for cattle may be used for late grazing in September or just prior to rehousing. In addition, in some circumstances not the entire herd of animals is treated with veterinary medicines. A recent survey of the use of parasiticides in cattle farms in the United Kingdom found that the proportion of dairy and beef cattle treated with parasiticide varied from 10% to 100%, although it was rare that the entire herd was treated at the same time ((Boxall et al., 2007). The same survey also found that the majority of farmers separated their treated and untreated cattle when they were released to pasture.

Persistence of residues will be heavily influenced by climatic conditions, differing between location and season, and affecting chemical degradation and dung degradation. For example, Halley et al. (1989a) found that the degradation of ivermectin would be in the order of 7 to 14 days under summer conditions, and in the order of 91–217 days in winter. The timing of application of manure or slurry to land may therefore be a significant factor in determining the subsequent degradation rate of a compound.

6.5.2 Dissipation and Transport in Soil Systems

When a veterinary medicine reaches the soil, it may partition to the soil particles, run off to surface water, leach to groundwater, or be degraded. Over time most compounds dissipate from the topsoil. The dissipation of veterinary drugs in soil has been the topic in a number of studies (e.g., Blackwell et al. 2007; Halling-Sørensen et al. 2005). The dissipation of veterinary antibiotics following application to soil can be variously due to biodegradation in soil or soil–manure mixtures, chemical hydrolysis, sequestration in the soil due to various sorptive processes, or transport to another environmental compartment.

6.5.2.1 Biotic Degradation Processes

The main mechanism for dissipation of veterinary medicines in soils is via aerobic biodegradation. Degradation rates in soil vary, with half-lives ranging from days to years (reviewed in Boxall et al. 2004; and see Table 6.5). Degradation of veterinary medicines is affected by environmental conditions such as temperature and pH and the presence of specific degrading bacteria that have developed to degrade groups of medicines (Gilbertson et al. 1990; Ingerslev and Halling-Sørensen 2001). As well as varying significantly between chemical classes, degradation rates for veterinary medicines also vary within a chemical class. For instance, of the quinolones, olaquindox can be considered to be only slightly persistent (with a half-life of 6 to 9 days), whereas danofloxacin is very persistent (half-life 87 to 143 days). In addition, published data for some individual compounds show that persistence varies according to soil type and conditions. In particular, diazinon

TABLE 6.5
Mobility and persistence of veterinary medicines, classification of persistence, and mobility

	Nonpersistent (DT_{50} < 5 days)	Slightly persistent (DT_{50} 5–21 days)	Moderately persistent (DT_{50} 22–60 days)	Very persistent (DT_{50} > 60 days)	Unknown
Very mobile (K_{oc} < 15)	Sulfamethazine				
Mobile (K_{oc} 15–74)		Metronidazole	Clorsulon Forfenicol		
Moderately mobile (K_{oc} 75–499)	Sulfadimethoxine	Olaquindox Piperonyl butoxide	Ceftiofur	Chlorfenvinphos Diclazuril (silty clay loam)	
Slightly mobile (K_{oc} 500–4000)	Tylosin (soil and manure)	Diazinon Tylosin (soil only) Emamectin benzoate		Eprinomectin Diclazuril (sandy loam and silt loam) Oxfendazole	Efrotomycin (loam, silt loam)
Nonmobile (K_{oc} > 4000)		Avermectin B1a (sandy loam soil)	Avermectin B1a (sandy soil) Deltamethrin	Albendazole Coumaphos Cypermethrin Danoflaxacin Doramectin Erythromycin Ivermectin Moxidectin Oxytetracycline Selamectin	Ciprofloxacin Efrotomycin (sandy loam, clay loam) Enrofloxacin Ofloxacin Tetracycline
Unknown K_{oc}				Sarafloxacin	

Source: Hollis (1991).

was shown to be relatively labile (half-life 1.7 days) in a flooded soil that had been previously treated with the compound, but was reported to be very persistent in sandy soils (half-life 88 to 112 days) (Lewis et al. 1993). Of the available data, coumaphos and emamectin benzoate were the most persistent compounds in soil (with half-lives of 300 and 427 days, respectively), whereas tylosin and dichlorvos were the least persistent (with half-lives of 3 to 8 days and < 1 day, respectively).

A number of suitable validated guideline methods developed for pesticide scenarios exist for examining degradation under aerobic, anaerobic, and denitrifying conditions. These may be a starting point for assessing veterinary medicines. An important question also to consider is the role of manure in soil systems in terms of degradation pathways and removal rates.

Manure amendment changes the properties of the soil system by increasing water content and organic carbon, and by modifying pH and the buffering capacity of the soil. Furthermore, inclusion of manure alters bacterial abundance and diversity in the topsoil. Whether changes in microbiological degradation pathways result from manure inclusion is not currently known. Initial laboratory-scale investigations suggest that manure inclusion up to 10% by weight does not affect the rate of degradation of tylosin, olaquindox, and metronidazole (Ingerslev and Halling-Sørensen 2001). But recent studies have shown that when manure is combined with soil, degradation may be enhanced for selected medicines such as sulfadimethoxine (Wang et al. 2006).

Compounds can be applied to the field in solid or slurried manure, with either a surface or subsurface application. No guidance exists on the methods to be used to evaluate veterinary medicine degradation in the field, but the practices employed in pesticide field dissipation studies may be used in this context, as the scenarios are very similar. It is important that the application method selected reflects common agronomic practice for the situation under consideration. Assessing antibacterial and fungicidal agents at unrealistically high spiking levels of the compounds may give false data on biotic removal due to bacteriostatic or bacteriocidal effects of tested compounds. Radiolabeled antimicrobial agents may also not be commercially available as they can be difficult to produce due to their semisynthetic origin.

Few studies have been carried out in the field, so limited data are available on veterinary medicine field dissipation (Kay et al. 2004; Halling-Sørensen et al. 2005; Blackwell et al. 2007).

6.5.2.2 Abiotic Degradation Processes

Depending on the nature of the chemical, other degradation and depletion mechanisms may occur, including soil photolysis, hydrolysis, and soil complex formation. The degradation products of both photolytic and hydrolytic degradation processes may undergo aerobic biodegradation in upper soil layers or anaerobic degradation in deeper soil layers. For many medicines, both hydrolysis and photolysis may be important dissipation pathways. Once manure is incorporated into the soil these processes are less important, but they may still be relevant in water.

ISO, OECD, and other standardizing bodies have developed appropriate methods for chemical substances for assessing hydrolysis, photolysis, and soil sorption. However, once again the influence of manure amendment should be considered for veterinary medicines, if appropriate.

6.5.2.3 Sorption to Soil

The degree to which veterinary medicines may adsorb to particulates varies considerably (Table 6.5), and this also affects the potential mobility of the compound. This can be influenced by the pH of the soil, depending on the ionic state of the compound under consideration. Partition coefficients (K_D) range from low (0.6 l kg^{-1}) to high (6000 l kg^{-1}) adsorption (K_{oc}; the organic normalized partition coefficient ranges from 40 to 1.63×10^7 l kg^{-1}). In addition, the variation in partitioning for a given compound in different soils can be significant (up to a factor of 30 for efrotomycin).

The range of partitioning values can be explained to some extent by studies addressing the sorption of tetracycline and enrofloxacin. The results suggest that surface interactions of these compounds with clay minerals are responsible for the strong sorption to soils. The underlying processes are cation exchange (tetracycline at low pH) and surface complex formation with divalent cations sorbed at the clay surfaces (tetracycline at intermediate pH and enrofloxacin at high pH). This indicates that in order to arrive at a realistic assessment of the availability of these compounds for transport through the soil and uptake into soil organisms, soil chemistry may not be reduced to the organic carbon content but the clay content, the pH of the soil solution, and the coverage of the ion exchange sites need to be accounted for.

Manure and slurry may also alter the behavior and transport of veterinary medicines. Studies have demonstrated that the addition of these matrices can affect the sorption behavior of veterinary medicines and that they may affect persistence (Boxall et al. 2002; Thiele Bruhn and Aust 2004). These effects have been attributed to changes in pH or the nature of dissolved organic carbon in the soil/manure system.

Guideline methods applicable to veterinary medicines are published by several regulatory bodies (e.g., the ISO and OECD). A substantial number of published data on sorption coefficients can be found in the open literature and are often higher than expected from their lipophilicity (e.g., tetracyclines and quinolones; Tolls 2001). Thus quantitative structure-activity relationships based on parameters such as K_{ow} can overestimate mobility. Coefficients are concentration dependent, and high spiking concentrations may give unrealistic results.

6.5.3 Bound Residues

Nonextractable residues are formed in soils during the application of pesticides (Führ 1987; Calderbank 1989). Sequestered residues have the potential to be transported to subsurface water through preferential flow. More detailed experiments

are needed to understand these mechanisms for veterinary medicines, and the VICH guidelines indicate that a case-by-case evaluation has to be conducted. The ionic nature of veterinary medicines makes it difficult to predict their behavior under all conditions. Time-dependent sorption appears to be a very important mechanism of removal for certain compounds (e.g., tetracyclines). Bound residues are also an important aspect in effect studies and are dealt with in Chapter 7 of this book.

The mechanisms by which residues become bound are numerous and relate to both the target molecule and the specific soil type. Characterization of bound residues by extraction with organic solvents, treatment with acid–base reflux procedures, and enzymes may assist in defining the fraction of the soil to which the residue is associated. However, these procedures can only be effectively conducted where the parent compound was applied in a radiolabeled form, and such analyses will not necessarily provide information on the structure of the residues released. Residues from biomass or highly degraded compounds are not considered bound residues by the International Union of Pure and Applied Chemistry (IUPAC) definition of pesticides (Roberts 1984). However, bound residues cannot be distinguished from biogenic residues, because the chemical structures of the residues are not known. The chemical reactivity of an active compound or of a metabolite governs the formation of bound residues, whose levels may range from 7% to 90% of the quantity applied (Calderbank 1989). Many pesticides are partially degraded, and the metabolites are involved in the formation of bound residues (Hsu and Bartha 1976).

Only a few studies have addressed the question of bound residues of veterinary medicines. Chander et al. (2005) investigated the process by sorbing various amounts of tetracycline or tylosin on two different textured soils (Webster clay loam [fine-loamy, mixed, superactive, mesic Typic Endoaquolls] and Hubbard loamy sand [sandy, mixed, frigid Entic Hapludolls]), incubating these soils with three different bacterial cultures (an antibiotic-resistant strain of *Salmonella* sp. [*Salmonella*^{R}], an antibiotic-sensitive strain of *Salmonella* sp. [*Salmonella*^{S}], and *Escherichia coli* ATCC 25922), and then enumerating the number of colony-forming units relative to the control. Soil-adsorbed antibiotics were found to retain their antimicrobial properties because both antibiotics inhibited the growth of all three bacterial species. Averaged over all other factors, soil-adsorbed antimicrobial activity was higher for Hubbard loamy sand than for Webster clay loam, most likely due to the higher affinity (higher clay content) of the Webster soil for antibiotics. Similarly, there was a greater decline in bacterial growth with tetracycline than with tylosin, likely due to greater amounts of soil-adsorbed tetracycline and also due to the lower minimum inhibitory concentration of most bacteria for tetracycline compared with tylosin. The antimicrobial effect of tetracycline was also greater under dynamic than static growth conditions, possibly because agitation under dynamic growth conditions helped increase tetracycline desorption and/or increase contact between soil-adsorbed tetracycline and bacteria. Chander et al. (2005) concluded that even though antibiotics are tightly adsorbed by clay particles, they are still biologically active and may influence the selection of antibiotic-resistant bacteria in the terrestrial environment.

6.6 UPTAKE BY PLANTS

The potential for medicines to be taken up by plants has also been considered (e.g., Migliore et al. 1996, 1998, 2000; Forni et al. 2001, 2002; Kumar et al. 2005; Boxall et al. 2006). Uptake of fluoroquinolones, sulfonamides, levamisole, trimethoprim, diazinon, chlortetracycline, and florfenicol has been demonstrated experimentally. Uptake can differ according to the crop type. For example, Boxall et al. (2006) demonstrated that florfenicol, levamisole, and trimethoprim were taken up by lettuce, whereas diazinon, enrofloxacin, florfenicol, and trimethoprim were detected in carrot roots. Kumar et al. (2005) showed in a greenhouse study in which manure was applied to soil that the plants absorbed antibiotics present in the manure. The test crops were corn (*Zea mays*), green onion (*Allium cepa*), and cabbage (*Brassica oleracea*). All three crops absorbed chlortetracycline but not tylosin. The concentrations of chlortetracycline in plant tissues were small (2 to 17 ng g^{-1} fresh weight), but these concentrations increased with increasing amounts of antibiotics present in the manure. Such studies point out the potential risks to humans and wildlife associated with consumption of plants grown in soil amended with antibiotic-laden manures.

6.7 MODELS FOR ESTIMATING THE CONCENTRATION OF VETERINARY MEDICINE IN SOIL

From the above, it is clear that the exposure of the environment to a veterinary medicinal product is determined by a range of factors and processes. When assessing the environmental risks posed by a new product, models and model scenarios are typically used to estimate the level of exposure. For environmental risk assessment purposes, these modeling approaches must be responsive to regional soil and climate conditions, as well as manure storage and handling conditions that can influence the persistence of excreted residues. Regional agronomic considerations and regulations that proscribe and constrain manure application rates, timing, and method must likewise be considered. Some emission scenarios (e.g., sheep dipping) are very country or even region specific. Currently employed terrestrial assessment models generally assume that residues, following excretion, are uniformly distributed in the terrestrial environment. In fact the distribution may be quite patchy, particularly in the case of dung that is excreted by animals on pasture. Currently, terrestrial exposure assessments contain the following elements:

- Information on the treatment of terrestrial animals
- Factors influencing the uptake and excretion of veterinary medicines by the animals
- Factors affecting how much residue reaches the land
- Factors affecting dissipation once the substance reaches the soil

In the following sections, we describe these models in more detail.

6.7.1 Intensively Reared Animals

For intensively reared animals that are housed indoors throughout the production cycle, treatment with the veterinary medicine is carried out in housed animals, and the active residue is excreted indoors and incorporated into the slurry or farmyard manure. This active residue reaches the environment when the manure from the stable is spread onto land. A number of models have been proposed to enable the calculation of the concentration of a veterinary medicine in soil after spreading manure from treated animals, based on a fixed amount of manure that can be spread on an area of land, and then incorporation to a uniform depth of soil. The mass of manure spread per unit area is usually controlled by the amount of nitrogen or, less frequently, by the amount of phosphorus in the manure.

The first of these methods was developed by Spaepen et al. (1997). In this method the concentration of the veterinary medicine in manure is calculated after treatment of the housed animals. In addition to the dose and duration of treatment, the calculation requires information on the body weight of the individual animal at treatment, the number of animals kept in 1 stable or barn each year, and the annual output of manure from the stabled animal. Following calculation of the concentration of veterinary medicine in manure, the quantity of manure that is spread per hectare of land is determined. The rate is controlled by the nitrogen or phosphorus content of the manure, which is provided in the publication with default values for most of the other parameters. The PEC_{soil} is calculated by calculating the mass of veterinary medicine spread per hectare of soil divided by the weight of the soil in the layer into which the residue penetrated, plus the weight of the manure (Equations 6.1 to 6.4). The PEC_{soil} is an annual value. An evaluation of this method against measured concentrations for veterinary medicines in the field indicates that it is likely to produce conservative exposure estimates (Blackwell et al. 2005).

$$M = D \times BW \times T \times C \tag{6.1}$$

$$C_{\text{excreta}} = \frac{M}{P_{\text{excreta}}} \tag{6.2}$$

$$R_{\text{hectare}} = C_{\text{excreta}} \times \frac{170}{N_{\text{prod}}} \times P_{\text{excreta}} \tag{6.3}$$

$$\text{PEC}_{\text{soil}} = \frac{R_{\text{hectare}} \times 1000}{\left(\frac{5}{100} \times 1500 \times 10000\right) + \left(\frac{170}{N_{\text{prod}}} \times P_{\text{excreta}}\right)} \tag{6.4}$$

where

PEC_{soil} = predicted environmental concentration in soil (μg kg^{-1})
M = total dose administered (mg)
D = dosage used (mg kg^{-1} body weight d^{-1})
T = number of daily administrations in 1 course of treatment (days)
BW = animal body weight (kg)
C = number of animals raised per place per year
$C_{excreta}$ = concentration of active in excreta (mg kg^{-1})
$P_{excreta}$ = excreta produced per place per year (kg y^{-1})
N_{app} = nitrogen application rate (kg ha^{-1} y^{-1})
N_{prod} = nitrogen produced per place per year (kg N y^{-1})
1500 = soil bulk density (kg m^{-3})
10 000 = area of 1 hectare (m^2 ha^{-1})
5 = depth of penetration into soil (cm)
$R_{hectare}$ = mass of active spread per hectare (mg ha^{-1})
1000 = conversion factor (μg kg^{-1})

A similar method to calculate the PEC_{soil} was developed by the Animal Health Institute (AHI) and Center for Veterinary Medicine (CVM) in the United States (Robinson personal communication 2006). In this method the concentration of the drug in manure is calculated by multiplying the dose per animal (mg kg^{-1} body weight) by the number of treatments, and dividing by the total amount of manure produced in the production period. The PEC_{soil} is calculated by multiplying the concentration of the drug in manure by the amount of manure allowed to be spread per hectare (a fixed value for each of cattle, pigs, and poultry) and dividing by the mass of 1 hectare of soil mixed to a depth of 15 cm. The value is an annual value.

Montforts (1999) developed a method specifically for the situation in the Netherlands, where the quantity of manure that can be spread onto land is restricted by the amount of phosphorus allowed.

The method of Montforts and Tarazona (2003) assumes that the average storage time for manure on the farm before spreading is 30 days. It is assumed that the treatment of the animals with the product occurs during the 30-day storage period and then the manure is spread onto land to comply with the nitrogen standard. This method does not consider the number of animals kept per stable unit per year (Equation 6.5).

$$PEC_{soil} = \left(\frac{D \times T \times BW \times 170}{1500 \times 10000 \times 0.05 \times N} \right) \times 1000 \tag{6.5}$$

where

PEC_{soil} = predicted environmental concentration in soil (μg kg^{-1})
D = dosage used (mg kg^{-1} body weight d^{-1})

T = number of daily administrations in 1 course of treatment (days);
BW = animal body weight (kg)
170 = EU nitrogen-spreading limit (kg N ha^{-1} y^{-1})
1500 = soil bulk density (kg m^{-3})
10 000 = area of 1 hectare (m^2 ha^{-1})
0.05 = depth of penetration into soil (m)
N = nitrogen produced in 30 days (kg N)

A fifth method has been proposed recently in a draft guideline published for consultation by the Committee for Medicinal Products for Veterinary Use (CVMP 2006; see Equation 6.6). The method is again based on spreading manure according to the nitrogen content of the manure. The number of animals occupying a stable unit over the year is also considered.

$$\mathrm{PEC}_{\mathrm{soil}} = \left(\frac{D \times T \times BW \times C \times 170 \times F}{1500 \times 10000 \times 0.05 \times N \times H} \right) \times 1000 \tag{6.6}$$

where

$\mathrm{PEC}_{\mathrm{soil}}$ = predicted environmental concentration in soil (μg kg^{-1})
D = dosage used (mg kg^{-1} body weight d^{-1})
T = number of daily administrations in 1 course of treatment (days)
BW = animal body weight (kg)
C = number of animals raised per place per year
170 = EU nitrogen-spreading limit (kg N ha^{-1} y^{-1})
F = fraction of herd treated (value between 0 and 1)
1500 = soil bulk density (kg m^{-3})
10 000 = area of 1 hectare (m^2 ha^{-1})
0.05 = depth of penetration into soil (m)
N = nitrogen produced in 1 year (kg N y^{-1})
H = housing factor (either one for animals housed throughout the year or 0.5 for animals housed for only 6 months)
1000 = conversion factor (μg kg^{-1})

These 5 methods of calculating a $\mathrm{PEC}_{\mathrm{soil}}$ value can be compared using a standard treatment scenario of a hypothetical veterinary medicine dosed at 10 mg kg^{-1} body weight for 5 days. The $\mathrm{PEC}_{\mathrm{soil}}$ values resulting from the different calculation methods are given in Table 6.6. In general, the $\mathrm{PEC}_{\mathrm{soil}}$ values calculated using the phosphorus standard to control the amount of manure spread onto land are the lowest. The method of Montforts and Tarazona (2003) gives the highest values when used to calculate the PEC for animals that have a single production cycle per year.

A comparison of predicted concentrations, obtained for the Spaepen, CVMP, and Montforts and Tarazona models, with measured environmental concentra-

TABLE 6.6
Comparison of predicted environmental concentration in soil (PEC_{soil}) values using different calculation methods obtained for a hypothetical veterinary medicine dosed at 10 mg kg^{-1}

	PEC_{soil} value (μg kg^{-1})			
Calculation method	**Fattening pig**	**Dairy cow**	**Beef bullock**	**Broiler**
Spaepen et al. (1997)	389	69	104	877
Montforts (1999)	297	18	40	148
US AHI/CVM Robinson (2006)	692	94	45	323
Montforts and Tarazona (2003)	1228	983	1338	567
Committee for Medicinal Products for Veterinary Use (2006)	269	147	214	374

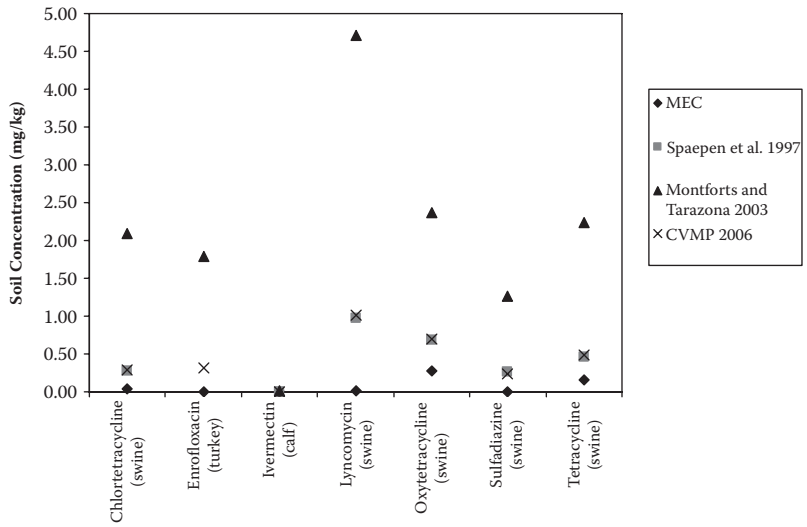


FIGURE 6.3 Measured and predicted environmental concentrations (MEC and PEC) for a range of veterinary medicines. *Source*: Measured concentrations from Hamscher et al. (2005), Boxall et al. (2006), and Zilles et al. (2005).

tions for a range of veterinary medicines (Figure 6.3) demonstrates that all of the models are likely to overestimate concentrations of veterinary medicines in the soil environment and that the Montforts and Tarazona (2003) model will greatly overestimate concentrations.

6.7.2 Pasture Animals

Calculation of the PEC_{soil} for pasture animals is dependent on the number of animals kept on a given area of land. This parameter is known as the stocking density and is expressed in animals per hectare. The PEC_{soil} is the total mass of active substance administered divided by a mass of soil of 750 000 kg (assuming penetration to 5 cm). It is assumed that the residue is evenly distributed over the pasture. This model was proposed by the CVMP in their recently published draft guidance (CVMP 2006). Using the model treatment regime of 5 days of treatment of 10 mg kg^{-1} body weight, the PEC_{soil} values for dairy cattle (body weight 500 kg and stocking density 3.33 animals per hectare) and beef cattle (body weight 350 kg and stocking density 6.4 animals per hectare) are 111 μg kg^{-1} and 149 μg kg^{-1}, respectively.

In the above calculations it is assumed that the veterinary product is excreted and distributed evenly over the pasture. For many products used to treat parasites, a significant proportion of the medicine is excreted in feces. For this reason it is necessary to calculate a PEC value for the dung in order to examine the effect of this residue, in particular on dung insects. A method of calculating the PEC in dung has been proposed by the CVMP (CVMP 2006) that can be used in the absence of any excretion data, but can also be refined if excretion data are available. In this method the highest fraction of the dose excreted daily in dung (or the total dose if there is no further information) is calculated and divided by the mass of dung excreted daily. For the above example, if a single day's treatment of 10 mg kg^{-1} was excreted in feces, over the following 24 hours the PEC in dung would be 96 mg kg^{-1}, as 52 kg of dung is assumed to be excreted by a dairy cow in 24 hours.

6.7.3 PEC Refinement

The present guidelines for environmental risk assessments (especially VICH Phase II and the VICH-EU-TGD; see Chapter 3) underline the use of a "total residue approach" as the first step in estimating environmental concentrations. Under these conditions no adjustment is recommended in which available metabolism and excretion data can be used. However, exceptions may be appropriate when substantial metabolism can be demonstrated (i.e., all individual excreted metabolites are less than 5% of applied dose). In some cases it may be appropriate during the tiered risk assessment procedure to utilize metabolism data to refine PEC_{soil} or PEC_{dung}. For example, if metabolites accumulate in the animal this may reduce initial concentrations in the collected manure or the excreted dung. Consequently, after distribution of feces/manure onto land, the original PEC_{soil} can also be refined.

A different refinement may be carried out for the PEC_{dung}, dealing either with excretion data or with knowledge of which fractions are excreted via urine and which are excreted via feces. Exposure scenarios may then be refined to consider direct soil influence through urine and the residues primarily associated with dung.

6.8 RESEARCH NEEDS

Reliable methods for evaluating potential environmental exposure require both experimental data for a number of key endpoints (e.g., DT_{50} values, K_{oc}, and water solubility) as well as sophisticated modeling tools for predicting reliable and realistic environmental concentrations.

The following research needs have been identified:

- Development of clear guidance specific to veterinary medicines for laboratory and field-based methods for the evaluation of degradation and dissipation: these should take into account agronomic practice when appropriate (e.g., the addition of manure or slurry).
- Field-based validation of PEC modeling methods needs to be conducted, as there is a perception that existing methods may be too conservative and unrealistic.
- The impact of different storage/composting conditions on the degradation of veterinary medicines needs to be better understood and investigated.
- Evaluation of the potential for desorption needs to be better understood and studied.
- Exposure scenarios following the application of combination products need to be considered.

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Environmental Risk Assessment of Ivermectin: A Case Study

Markus Liebig,† Álvaro Alonso Fernandez,‡ Elke Blübaum-Gronau,§ Alistair Boxall,‖ Marvin Brinke,# Gregoria Carbonell,‡ Philipp Egeler,† Kathrin Fenner,††,‡‡ Carlos Fernandez,‡ Guido Fink,§ Jeanne Garric,§§ Bent Halling-Sørensen,‖‖ Thomas Knacker,† Kristine A Krogh,‖‖ Anette Küster,## Dirk Löffler,§ Miguel Ángel Porcel Cots,‡ Louise Pope,‖ Carsten Prasse,§ Jörg Römbke,† Ines Rönnefahrt,## Manuel K. Schneider,†† Natascha Schweitzer,† José V Tarazona,‡ Thomas A Ternes,§ Walter Traunspurger,# Anne Wehrhan,## and Karen Duis†*

†ECT Oekotoxikologie GmbH, Böttgerstrasse 2-14, D-65439 Flörsheim/Main, Germany
‡Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Ctra La Coruña km 7.5, E-28040 Madrid, Spain
§Federal Institute of Hydrology, Am Mainzer Tor 1, D-56068 Koblenz, Germany
‖Central Science Laboratory (CSL), Sand Hutton, York YO41 1LZ, United Kingdom
#Department Animal Ecology, University of Bielefeld, Morgenbreede 45, D-33615 Bielefeld, Germany
††Swiss Federal Institute of Aquatic Science and Technology (Eawag), Überlandstrasse 133, CH-8600 Dübendorf, Switzerland
‡‡Institute of Biogeochemistry and Pollutant Dynamics (IBP), ETH Zürich, Universitätsstrasse 16, CH-8092 Zürich, Switzerland
§§Cemagref, UR BELY, Laboratory of Ecotoxicology, 3 bis, Quai Chauveau, CP 220, F-69336 Lyon Cedex 09, France
‖‖Faculty of Pharmaceutical Sciences, Copenhagen University, Universitetsparken 2, DK-2100 Copenhagen, Denmark
##Federal Environment Agency (UBA), Postfach 1406, D-06813 Dessau, Germany

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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

Keywords: Environmental risk assessment Fate and effects assessment Parasiticides Tiered approach Veterinary pharmaceuticals

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

* To whom correspondence may be addressed: m-liebig@ect.de
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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.

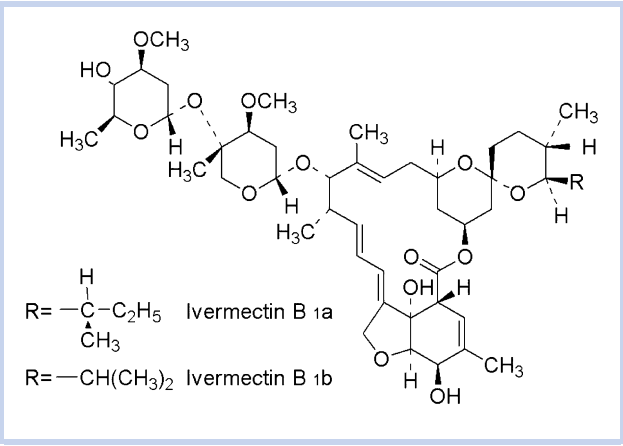

Figure 1. Chemical structure of ivermectin.

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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