Sarcoptic Mange Treatment in Wombats:
Environmental Risk Assessment Report
CONTEXT NOTE[1]

The attached report, *Sarcoptic Mange Treatment in Wombats: Environmental Risk Assessment,* is a desktop assessment of the potential for environmental effects of flurulaner, ivermectin and moxidectin associated with the field treatment of sarcoptic mange in wombats. The report is an independent body of work undertaken by Arcadis Australia Pacific Pty Ltd, funded by Wildlife Health Australia's One Health Investigation Fund.

The report's environmental risk assessment, modelling and recommendations are based on current knowledge of the properties of these drugs, their modes of use to treat sarcoptic mange in wombats, and published information on their direct toxicological effects on non-target species. The report may be used as a reference tool to:

i) guide field research of environmental impacts of wombat antiparasitic treatment
ii) inform local assessment of risk (including managing drug use in highly sensitive environments)
iii) inform the development of specific jurisdictional recommendations on wombat antiparasitic treatment regimes.

Please note that this report represents the findings of a desktop exercise and literature review. Information on the relative assessment of antiparasiticals as a 'hazard' (ie absolute risk as modelled in the report) may not reflect the local risks associated with antiparasitic use in wombats in the field.

There are other considerations to antiparasitic use in wombats in the field that will contribute to decision making for wombat dosage and treatment regimes, which are beyond the scope of the report.

Key findings of the assessment are summarized below and in the Executive Summary of the report:

- the main routes of drugs potentially entering the environment after antiparasitic treatments of wombats are: accidental spillage, excess run-off or shake-off by the wombat after application, excretion in faeces, and washoff from the terrestrial environment into waterways. Likely routes of entry are summarised in Figure 5-1 on page 11 of the report.
- from an environmental toxicity perspective, the duration of drug persistence in the environment appears comparatively more important than differences in drug volume and retreatment frequency.
- because large-volume applications of moxidectin lead to direct entry of the drug into the environment (i.e., excess treatment runoff, and wombat shake-off), using a minimum volume that is most consistently therapeutic to the wombat is recommended.
- based on available evidence, moxidectin has a shorter period of persistence in the terrestrial environment, with lower toxicity metabolites, than fluralaner (the same feature that extends the treatment/efficacy period for fluralaner in wombats).
- both moxidectin and fluralaner treatments have potential to be toxic to dung fauna, although research is needed so that comparisons of these drugs can be reliably made. The duration of drug persistence in the environment may also influence potential toxic impacts.
- moxidectin may be more toxic than fluralaner in aquatic environments, although research is needed so that comparisons can be more reliably made, and moxidectin may breakdown in aquatic environments more rapidly. Management of potential drug entry into waterways is therefore recommended for all treatments.
- this expert assessment evaluates the potential for toxic effects of wombat treatments in the environment. The absolute risks of both moxidectin and fluralaner, in terms of detectable impacts that are observed in the environment, is yet to be assessed.

[1] This context note has been compiled by Wildlife Health Australia in consultation with the report author, and government and non-government stakeholders involved in the field treatment of wombats for sarcoptic mange.

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Environmental Risk Assessment

March 2024

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Contents

Tables

Figures

Appendices

Acronyms and Abbreviations

Acronym	Definition
ADME	Absorption, distribution, metabolism, and excretion
APVMA	Australian Pesticides and Veterinary Medicines Authority
BCF	Bioconcentration factor
CAS RN	Chemical Abstracts Service Registry Number
CEM	Conceptual Exposure Model
DT50	Degradation time 50%. Time for 50 percent of the chemical to degrade
dw	Dry weight
EC50	Effective concentration, 50%. The concentration that causes an adverse effect to 50% of the tested organisms.
fw	Fresh weight
GABA	Gamma-aminobutyric acid
Koc	Organic-carbon partition coefficient
Kow	Octanol-water partition coefficient
LC50	Lethal concentration, 50%. The concentration that is lethal to 50% of the tested organisms
LD50	Lethal dose, 50%. The dose that is lethal to 50% of the tested organisms
LOEC	Lowest observed effect concentration
NOAEC	No observed adverse effect concentration
NOEC	No observed effect concentration
NOEL	No observed effect level
PEC	Predicted environmental concentration
PBT	Persistent, bioaccumulative, toxic
TOC	Total organic carbon
UNAF	Union Nationale de L'apiculture Francaise
VICH	Veterinary International Conference on Harmonization
WHA	Wildlife Health Australia

Executive Summary

Arcadis Australia Pty Ltd (Arcadis) was engaged by Wildlife Health Australia (WHA) to undertake a review of the environmental effects of the off-label use of the anti-parasitical chemicals fluralaner, ivermectin and moxidectin in the treatment of sarcoptic mange in wombats.

The anti-parasitical chemicals fluralaner (in Bravecto®) and moxidectin (in Cydectin®) are applied topically to free-ranging wombats, whereas ivermectin is generally used by injection to captive wombats or those under rehabilitation. Bravecto® and Cydectin® are veterinary products, registered for use in domestic animals such as dogs and cattle. Treatment volumes of Bravecto® for wombats range from 3.6 – 5 millilitres (mL) (40 – 56 milligrams (mg) fluralaner / kilograms (kg) wombat[1] / treatment), whereas relatively large volumes of Cydectin® may be used to treat wombats (20 – 200 mL per treatment; 4 – 40 mg moxidectin / kg wombat[1] / treatment). Higher treatment volumes can result in transfer of the active chemical(s)to the environment through spillages, run-off and and shake-off from the wombat and/or through residues in urine/dung and subsequent leaching into soil and water.

The objective of this review was to estimate the potential environmental effects (aquatic and terrestrial) from the treatment of free-ranging wombats using fluralaner, ivermectin and moxidectin (collectively referred to as the treatment chemicals, or chemicals under consideration) and provide recommendations that may inform appropriate treatment protocols that □inimize environmental risk.

The scope of work involved review of publicly available information on the physico-chemical properties of, and environmental data on, the three treatment chemicals stated above relevant to the project objective, and identification of data gaps and uncertainties in the available information that could inform additional research and/or data collection.

The treatment chemicals under consideration generally report low toxicity to birds and mammals, but high toxicity to aquatic organisms (fish, aquatic invertebrates and/or sediment dwellers) and dung (faecal) fauna. Whilst the properties of the treatment chemicals indicate a low likelihood of leaching from soil and dung, aquatic toxicity can occur at nanogram per litre (i.e., very low) concentrations, hence minimising the potential transfer of the chemicals to waterways is a key consideration. There are gaps in the understanding of the migration of the treatment chemicals in the environment and potential uptake by, and effects on, plants, and effects on bees and soil microorganisms. There is some evidence to indicate transfer to the nests of birds (and subsequent juvenile bird exposure), as well as plants growing adjacent to areas where animals have been treated.

A review of the available data indicates that moxidectin in Cydectin® has shorter degradation times in soil and water compared to fluralaner (in Bravecto®), and also appears to have relatively low toxicity to dung fauna. In addition, there is some evidence to indicate that the proportion of inactive metabolites in dung is higher for moxidectin than fluralaner, at least for some animals. For these reasons, moxidectin presents a lower risk to the environment than fluralaner, on a direct chemical-by-chemical comparison basis. This is the case even though greater volume losses of Cydectin® to the environment are expected compared with Bravecto®, because the concentration of fluralaner in Bravecto® is much higher than the concentration of moxidectin in Cydectin®.

Due to the excretion of un-metabolised treatment chemicals in wombat dung, the treatment of sarcoptic mange in free-ranging wombats will inevitably result in the transfer of some of the chemicals to the environment, regardless of which chemicals are used, or the treatment method. A key factor in mitigating environmental risk is associated with the applied dose and the application method; that is, adoption of the

[1] Assuming a 25 kg wombat

minimum effective dose to reduce the likelihood of spillage, as well as run-off and shake-off from treated wombats. Topical treatment volumes of approximately 20 mL or less appear less likely to result in environmental transfer through run-off and shake-off than higher treatment volumes. Ivermectin, being an injectable, presents a negligible risk of transfer to the environment during application, but is expected to be present in dung, and is highly toxic to dung fauna.

Environmental monitoring studies of residual concentrations of the treatment chemicals in dung, soil, plants and water in or near treatment areas of free-ranging wombats would provide additional information beneficial to understanding how the treatment chemicals behave in the environment, and whether they transfer to other environmental matrices (such as plants) or migrate away from treatment areas and wombat toileting areas. The results of these studies could then further inform treatment protocols and restrictions associated with sarcoptic mange in free-ranging wombats.

1 Introduction

Arcadis Australia Pacific Pty Ltd (Arcadis) was engaged by Wildlife Health Australia (WHA) to undertake a review of the environmental effects of the off-label use of the anti-parasitical chemicals fluralaner, ivermectin and moxidectin in the treatment of sarcoptic mange in free-ranging wombats.

Sarcoptic mange is a skin infection in mammals that is caused by a burrowing parasitic mite, *Sarcoptes scabiei*. It affects more than 100 mammalian species worldwide, including humans and dogs. The disease is referred to as scabies in humans and mange in other species. Of the Australian native mammals known to be affected by mange, wombats appear to be the most impacted. Both bare-nosed and southern hairy-nosed wombat populations are affected throughout their range (Old et al., 2021). Wombat burrows are suspected to support mange survival and transfer between wombats (DNRE, 2022; Browne et al., 2021).

Mange infection in an animal can result in aggressive scratching, hair loss, skin thickening and crusting, skin discoloration, open wounds (from scratching), weight loss, and in most cases, death (as a result of secondary infection and suppressed immune system) (DNRE, 2022).

Arcadis understands that treatment of sarcoptic mange in free-ranging wombats can involve the topical application of veterinary products such as Bravecto® (containing fluralaner) and Cydectin® (containing moxidectin), which are registered for use in domestic animals such as dogs and cattle. In some cases, relatively large volumes of the products (100 – 200 mL) may be used to treat individual wombats, and some of the active chemical(s) may enter the environment through run-off and shake-off from the wombat and/or through residues in urine/dung and subsequent leaching into soil and water. While the veterinary chemicals are registered in Australia for various uses, there is a lack of understanding of potential environmental impacts associated with the off-label use in free-ranging wombats.

2 Objective and Scope of Work

The objective of this project is to:

- estimate the potential environmental effects (aquatic and terrestrial) of fluralaner, ivermectin and moxidectin associated with the treatment of mange in wombats[2];
- provide recommendations that may inform appropriate protocols for the use of fluralaner and moxidectin in sarcoptic mange treatment of free-ranging wombats so as to minimise environmental risk.

The scope of work involves the following:

- liaison with WHA and subject matter experts to establish the off-label use profile(s), including volumes applied to free-ranging wombats, method(s) of application (e.g., at burrow entrances, on open ground etc), observations of run-off, field observations following application, etc;
- assessment of field analytical data and observations, where available, in areas where wombats have been treated (e.g., concentrations in dung, concentrations in soil or water);
- review of publicly available guidelines[3] for treatment of mange in wombats;

[2] Both fluralaner and moxidectin are applied topically for the treatment of mange in free-ranging wombats; whereas ivermectin is an injectable, used for the treatment of mange in captive wombats.
[3] Guidelines for the treatment of Australian wildlife with sarcoptic mange. Part 1 – Treatment Guidelines and Part 2 – Literature Review. July 2021, University of Melbourne and University of Tasmania.

- review of publicly available information on the physico-chemical properties of, and environmental data on, ivermectin, moxidectin and fluralaner relevant to understanding the environmental transport and fate of these chemicals in the treatment of sarcoptic mange in free-ranging wombats;
- review and collation of publicly available information on the environmental effects (aquatic and terrestrial) of ivermectin, moxidectin and fluralaner in the context of the treatment of sarcoptic mange in free-ranging wombats;
- development of a conceptual exposure model (CEM) that describes the transport and exposure pathways of ivermectin, moxidectin and fluralaner in the environment, as well as potential receiving environments and ecosystems;
- collation of ecotoxicity endpoints for ivermectin, moxidectin and fluralaner protective of the environmental receptors in the CEM (where possible);
- based on the above, provision of recommendations associated with the use of ivermectin, moxidectin and fluralaner in free-ranging wombats, that may support future development of protocols and associated risk communication materials for wildlife managers;
- identification of data gaps and uncertainties in the use profile, environmental effects, CEM and ecotoxicity endpoints, and provide potential approaches to filling the data gaps;
- provision of the findings of the scope of work in one draft and one final report.

3 Treatment

Treatment of sarcoptic mange in free-ranging wombats involves topical application of the anti-parasitic chemicals fluralaner (the active constituent in Bravecto® spot-on for dogs) or moxidectin (the active constituent in Cydectin®). Veterinarians treating mange in captive / in-care wombats also use injectable anti-parasiticals (e.g., subcutaneous ivermectin or moxidectin) at recommended livestock doses, however injection-based delivery methods have reduced practicability for free-ranging wombats (Skerratt et al., 2021).

Community groups involved in wombat welfare and conservation and mange treatment advocate for the delivery of topical anti-parasitical chemicals via non-invasive methods that avoid the need to capture affected wombats (Bains et al., 2022). Two main methods that are currently adopted: the “burrow flap” method and the “pole and scoop” technique (Old et al., 2021). The “burrow-flap” method involves installation of a flap with a treatment reservoir at a wombat’s burrow entrance. When a wombat pushes through the flap, the chemical is indirectly delivered onto the wombat’s back. The “pole and scoop” technique involves directly applying the chemicals, via a long pole, to approachable wombats (Old et al., 2021). Videos of these two delivery methods are shown on the Mange Management website (Mange Management , 2023).

There are difficulties with both the “burrow flap” and “pole and scoop” approaches, including identifying the specific wombats for treatment, as well as ensuring adequate drug absorption following topical treatment. Bains et al. (2022) report that *in situ* treatments can fail due to “run off” of the formulation following treatment, and poor penetration of the chemicals through fur and affected skin. Anecdotal information from wildlife carers and rehabilitators indicates that with increasing volumes of applied product, a significant volume is lost via run-off and through wombats shaking some of the product off following application. Estimated run-off/shaking losses for a treatment volume of 100 mL were 50%, however small treatment volumes (5 – 10 mL) are considered to have minimal run-off losses (S. Carver, *pers. comm*).

Other potential losses of product may occur through the delivery method. The pole and scoop method generally has a reasonably high level of accuracy, with failed delivery reported to be only about 10% of the time (S. Carver, *pers. comm*). With respect to burrow flaps, spills are reported to occur relatively frequently

(30-50% of the time for 20 mL volumes), with greater volumes of spillage with higher treatment volumes (S. Carver, *pers. comm*).

The Australian Pesticides and Veterinary Medicines Authority (APVMA) have provided minor-use permits for the treatment of mange in free-ranging wombats by moxidectin. For fluralaner and ivermectin, existing approvals have been adapted for wombats. The minor use permits, and in-field practices, involve application of higher volumes of product than label-specifications and higher volumes than previously anecdotally effective doses for wombats. This is to compensate for the likelihood that not all topically delivered medications will contact the skin and be absorbed (Skerratt et al., 2021), as well as the severity of the disease. Based on available information, some spillage of chemicals onto soils and vegetation may occur during treatment, with the additional potential for environmental exposure via run-off from wombats during rainfall, as well as leaching from soil and wombat dung to watercourses following rain events.

A summary of the permits/approvals and doses for the three active constituents is provided in **Table 3-1**.

Table 3-1 APVMA Permits and Approvals for Anti-parasitical use in wombats

Active constituent	Product	APVMA approval / Permit Number	Permitted / Recommended dose
Fluralaner	Bravecto® 1000 mg for Large Dogs 280 mg fluralaner/mL 3.57 mL/pipette	Recommended dose in APVMA minor use permit PER90837 v3 (2 March 2022 to 31 March 2024) (for application on wombats)	3.57 mL product / wombat*, up to 3 doses at intervals > 1 month = 1000 mg fluralaner / wombat / application = 40 mg fluralaner /kg wombat* / application
Fluralaner	Bravecto® 1400 mg Fluralaner spot-on Solution for Very Large Dogs 280 mg fluralaner/mL 5 mL/pipette	Recommended dose in APVMA approval 82795 / 141420 (for application on dogs)	5 mL product / dog = 1400 mg fluralaner / dog / treatment = 25 mg fluralaner/kg dog** = 56 mg fluralaner/kg wombat* / application
Ivermectin	ICON -F Broadspectrum antiparasitic injection for cattle 10 mg ivermectin/mL subcutaneous injection	Recommended dose in APVMA approval 90692/129798 (for application on cattle)	Recommended: 1 mL / 50 kg for cattle >650 kg = 0.2 mg ivermectin / kg If given to wombats: 0.2 - 0.4 mg/kg, weekly over 2 – 4 months (Skerratt et al., 2021)
Moxidectin	Cydectin® Pour-On for Cattle and Red Deer 5000 mg moxidectin/L	Recommended dose in APVMA minor use permit PER89040 v4 (15 June 2020 to 6 February 2024) (for application on wombats)	0.8 mL/kg, once weekly for up to 15 weeks. Max dose 20 mL/wombat*. = 4 mg moxidectin / kg wombat* / application
Moxidectin	Cydectin® Pour-On for Cattle and Red Deer 5000 mg moxidectin/L	Recommended dose in APVMA minor use permit PER90094 v 3 (9 August 2021 to 6 February 2024)	4 mL/kg every 5 – 7 days, for up to 5 doses Max dose 100 mL/wombat* = 20 mg moxidectin / kg wombat / application
Moxidectin	Cydectin® Pour-On for Cattle and Red Deer	Field example reported in Old et al. (2021)	200 mL/wombat initial dose, then 22 x 30 mL, 7

Active constituent	Product	APVMA approval / Permit Number	Permitted / Recommended dose
	5000 mg moxidectin/L		days apart; 161 day treatment period = 40 mg moxidectin / kg wombat initial application

* assuming 25 kg wombat. **assuming 55 kg dog

Table 3-2 below combines the various treatment regimes for fluralaner and moxidectin (as shown above in **Table 3-1**) with anticipated losses from run-off, shake-off and potential spills. Two scenarios are provided for fluralaner (based on the two treatment concentrations), and three scenarios are provided for moxidectin (based on two different application volumes and differing estimated losses to the environment from spills and run-off / shake-off). Ivermectin is not included in **Table 3-2** given the injectable route is not expected to result in run-off, shake-off or spillages during application.

Table 3-2 Summary of estimated losses to the environment from application of Bravecto® and Cydectin®

Product name	Bravecto®		Cydectin®		
Active chemical	**Fluralaner**		**Moxidectin**		
Product concentration (mg/L)	280000	280000	5000	5000	5000
Application volume (mL / treatment)	3.57	5	20	100	100
Intended application mass (mg / treatment)	1000	1400	100	500	500
Estimated run-off and shake-off percentage (%)	10	10	20	20	50
Number of treatments	3	1	15	5	5
Treatment interval	Monthly	NA	Weekly	5 – 7 days	5 – 7 days
Total treatment time	2 months	NA	3.5 months	20-28 days	20-28 days
Lost to the environment (mg / treatment)	100	140	20	100	250
Mass remaining on wombat (mg / treatment)	900	1260	80	400	250
Total lost to the environment via run-off/shake-off across all treatments (mg)	300	140	300	500	1250

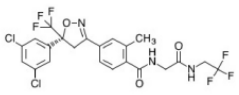
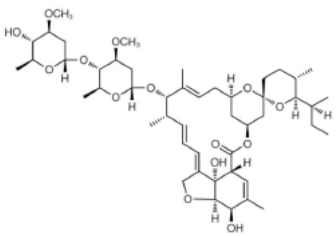
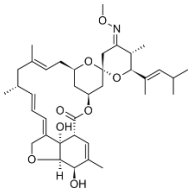
NA = Not applicable

4 Chemical Properties

4.1 Identity

A summary of information on the chemical identities of the active constituents is provided in Table 4-1.

Table 4-1 Chemical Identity Information

Property	Fluralaner	Ivermectin	Moxidectin
Product Name	Bravecto® spot-on for dogs	Virbamec Plus® injection endectocide and flukicide for cattle and ICON-F® Broadspectrum antiparasitic injection for cattle	Cydectin® pour-on for cattle and red deer
Concentration	280 mg/mL	10 g/L	5000 mg/L
CAS RN	864731-61-3	70288-86-7 (Ivermectin) 70161-11-4 (dihydroavermectin B1a) 70209-81-3 (dihydroavermectin B1b)	113507-06-5
Molecular formula	$C_{22}H_{17}Cl_2F_6N_3O_3$	$C_{48}H_{74}O_{14}$	$C_{37}H_{53}NO_8$
Molecular mass (g/mol)	556.3	875.1	639.8
Structural formula			

4.2 Chemistry and Mode of Action

Fluralaner is an isoxazoline class parasiticide, a systemic insecticide and acaricide, administered orally or topically. It acts as an antagonist on ligand-gated chloride channels (glutamate-gated and gamma-aminobutyric acid (GABA)-gated) in susceptible invertebrates (CoA, 2018). Fluralaner has high selectively for arthropods, and a favourable safety profile in vertebrates (CoA, 2018). It exists as a chiral molecule of *S* and *R* enantiomers, with the *S* enantiomer being the active component and the *R* enantiomer inactive (Evans et al., 2023). It is sold as a racemic mixture of both enantiomers.

Ivermectin is a macrocyclic lactone, consisting of a racemate of two enantiomers in an approximately 80:20 mixture of 22,23-dihydroavermectin compounds (avermectin B1a and avermectin B1b). Macrocyclic lactones are chemical derivatives of soil microorganisms belonging to the genus *Streptomyces*. Ivermectin is

particularly effective against gastrointestinal worms, most mites and some lice (PubChem, 2023b). It binds and activates glutamate-gated chloride channels, common to invertebrate muscle and nerve cells. This increases the flow of chloride ions and hyper-polarizes the cell membrane, resulting in paralysis and death. Ivermectin is safe for mammals because mammalian glutamate-gated chloride channels only occur in the brain and spinal cord, and the causative avermectins are large molecules that usually do not cross the blood-brain barrier. However, certain genetic lines of collies develop mild to moderate signs of toxicosis following ivermectin treatment (Lumaret et al., 2012).

Moxidectin is also a macrocyclic lactone and its mode of action is similar to other members of the macrocyclic lactone family, being interaction with the receptor channels for inhibitory neurotransmitters, competing with GABA-gated and glycine chloride channels, causing paralysis of some arthropods and nematodes (USDA, 2003; Awasthi, 2012). Moxidectin is effective against a wide range of adult and larval internal and external parasites, including roundworms, lungworms, mites and lice (USDA, 2003).

4.3 Metabolism and Elimination

Fluralaner is well distributed to tissues and appears to undergo enterohepatic recirculation, resulting in accumulation after daily oral administration (EMA, 2017b). Fluralaner does not undergo extensive metabolism, with the major route of elimination via the faeces: up to 49% in rats and 17% in dogs over a six day period, after the last administered dose (EMA, 2017b). Urinary elimination appears to be limited: up to 3.7% in rats and dogs (EMA, 2017b). In dogs (and other animals) multiple metabolites may be produced (EMA, 2017b). The half-life of fluralaner in wombats at the recommended dose (25 mg/kg) is 40.1 days and at a high wombat dose (85 mg/kg) is 166.5 days (Wilkinson et al., 2021). In dogs, the half-life has been reported at 14.9 days after a single dose at 25 mg/kg (Evans et al., 2023).

Macrocyclic lactones are also primarily excreted in the faeces (NRA, 1998). Ivermectin undergoes little metabolism and is excreted largely unchanged in the faeces of treated animals (Lumaret et al., 2012; de Souza & Guimaraes, 2022; Doran et al., 2024). Bains et al. (2022) recently reported that there are no pharmacokinetcs studies on ivermectin in wombats to date. In livestock, intravenous injection of ivermectin has a half-life of 32 – 65 h, whereas ivermectin as a pour-on has a half-life of 127.2 h (5.3 days) (Vercruysse & Rew, 2002).

Moxidectin is very lipophilic, so high concentrations of residues within treated animals are seen in fat, compared to other tissues (USDA, 2003). The primary excretion route for moxidectin is via the faeces, with some studies suggesting that most moxidectin is excreted as less active hydroxylated metabolites (USDA, 2003; Perez et al., 2001) however other studies (e.g., (Doran et al., 2024; Azfal et al., 1998) have indicated that moxidectin (and ivermectin) are primarily excreted un-metabolised in faeces. Pritchard et al. (2012) report that in cattle, around 13% of moxidectin is metabolised to mono-hydroxyl methyl derivatives (compared to 8% of ivermectin). The half-life of moxidectin in the southern hairy-nosed wombat at the recommended dose of 0.2 mg/kg (via sub-cutaneous injection) is approximately 5 days (Death et al., 2011).

5 Conceptual Exposure Model

For free-ranging wombats, excretion of the parent compounds in the dung provides the potential for secondary poisoning of ecological receptors via direct faecal exposure, as well as leaching or run-off from dung and from spillages at treatment locations. Urinary excretion appears to be limited and metabolism to more active compounds has not been reported. Should the compounds persist in soil and dung, this could lead to adverse effects in non-target species, such as terrestrial invertebrates and plants, as well as aquatic systems.

It has been noted since 1998, for example, that livestock treated with macrocyclic lactones will excrete a portion of the treatment dose in their dung, leading to potential effects in invertebrates that may utilise animal

dung as a food or breeding resource (NRA, 1998). Given the higher doses and reduced accuracy of treatment methods used for free-ranging wombats, off-target effects in the environment of the veterinary chemicals could be anticipated.

A conceptual exposure model of potential pathways of exposure to the environment from the off-label use of anti-parasitical chemicals is provided in Table 5-1 and Figure 5-1 below.

Table 5-1 Conceptual Exposure Model

Primary source	Application method	Primary migration pathway	Potentially exposed populations	Secondary migration pathway	Potentially exposed populations through secondary migration	Comment
Bravecto® - Fluralaner	Pole and scoop, and Burrow flap	Run-off /shake-off from wombat to soil and spills during application	Soil organisms – plants, terrestrial invertebrates, birds, reptiles and other mammals	Run-off to water	Aquatic organisms – plants, invertebrates, fish and frogs	Plausible
		Wash-off from wombat after rain to soil				Plausible
		Excretion via urine		-	-	Unlikely Urinary excretion is not considered to represent a significant secondary exposure pathway
		Excretion via dung	Soil organisms, particularly dung beetles and other invertebrates exposed to wombat dung.	Leaching from dung and run-off to soil and/or water	Soil and aquatic organisms – terrestrial and aquatic plants, terrestrial and aquatic invertebrates, fish and frogs	Plausible Excretion via dung is the main elimination route
		Loss of fur from treated animals	Birds and other animals that use animal hair for nesting	-	-	Pathway is plausible but effects likely negligible given the low density of treated free-ranging wombats, compared with treated livestock
	Burrow flap	Spillage at burrow entrance	Soil organisms – plants, terrestrial invertebrates, birds, reptiles and other mammals	Run-off to water	Aquatic organisms – plants, invertebrates, fish and frogs	Plausible Spills at burrow entrance could result in higher soil concentrations due to repeat applications

Primary source	Application method	Primary migration pathway	Potentially exposed populations	Secondary migration pathway	Potentially exposed populations through secondary migration	Comment
Ivermectin	Injection	Excretion via dung	Soil organisms, particularly dung beetles and other invertebrates exposed to dung	Leaching from dung and run-off to soil or water	Soil and aquatic organisms – terrestrial and aquatic plants, terrestrial and aquatic invertebrates, fish and frogs	Plausible
		Excretion via urine	Soil organisms – plants, terrestrial invertebrates, birds, reptiles and other mammals	-	-	Unlikely Urinary excretion is not considered to represent a significant secondary exposure pathway
Cydectin® pour-on - Moxidectin	Pole and scoop, and Burrow flap	Run-off / shake-off from wombat to soil and spills during application	Soil organisms – plants, terrestrial invertebrates, birds, reptiles and other mammals	Run-off to water	Aquatic organisms – plants, invertebrates, fish and frogs	Plausible
		Wash-off from wombat after rain to soil				Plausible
		Excretion via urine		-	-	Unlikely Urinary excretion is not considered to represent a significant secondary exposure pathway
		Excretion via dung	Soil organisms, particularly dung beetles and other invertebrates exposed to dung	Leaching from dung and run-off to soil or water	Soil and aquatic organisms – terrestrial and aquatic plants, terrestrial and aquatic invertebrates, fish and frogs	Plausible Excretion via dung is the main elimination route
		Loss of fur from treated animals	Adult and juvenile birds and other animals that use animal hair for nesting	-	-	Pathway is plausible but effects likely negligible given the low density of treated free-ranging wombats, compared with treated livestock

Primary source	Application method	Primary migration pathway	Potentially exposed populations	Secondary migration pathway	Potentially exposed populations through secondary migration	Comment
	Burrow flap	Spillage at burrow entrance	Soil organisms – plants, terrestrial invertebrates, birds, reptiles and other mammals	Run-off to water	Aquatic organisms – plants, invertebrates, fish and frogs	Plausible Spills at burrow entrance likely to result in higher soil concentrations due to repeat applications

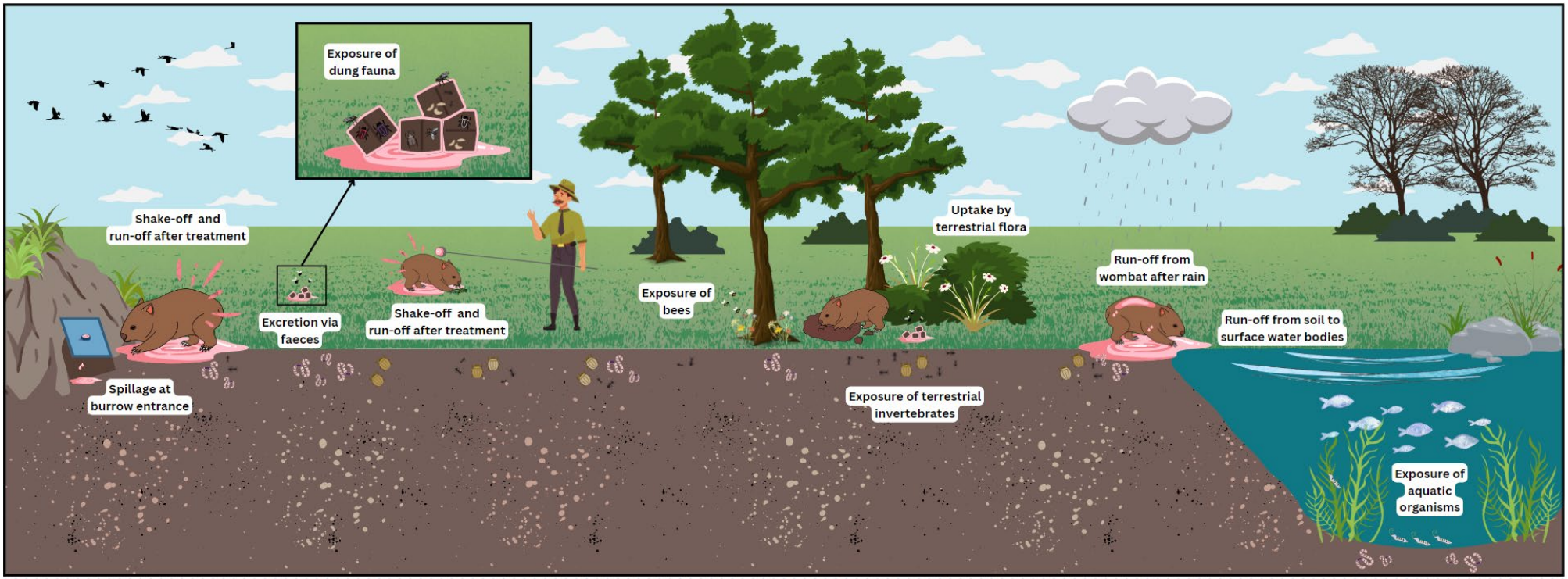


Figure 5-1 Conceptual Exposure Model

6 Fate and behaviour in the environment

Treatment of sarcoptic mange in wombats with anti-parasitical compounds can result in the veterinary chemicals entering the environment via two main pathways: spillage, run-off and shake-off during treatment (as reported by Old et al. (2021), and excretion via dung (Vercruysse & Rew, 2002), refer to Table 5-1). Minor pathways include run-off from treated wombats following rainfall and wash-off in (or transfer to) water following treatment. Urinary excretion does not appear to be a significant pathway of the chemicals to the environment.

In situations where the anti-parasitical compounds enter the environment, their fate and behaviour are determined by chemical properties (including **persistence** (P) and **bioaccumulation** (B) potential). Off-target risks are therefore identified by assessing chemical properties (including P and B) as well as **toxicity** (T).

DCCEEW (2022) recently published a set of PBT criteria for industrial chemicals (shown in **Table 6-1** below), which are also considered applicable to agricultural and veterinary chemicals (e.g., (Lee-Steere, 2009)).

Table 6-1 PBT Criteria

Characteristic	Matrix	Indicator and Threshold
Persistence	Water	Half-life ($T_{1/2}$) ≥ 60 days
	Soil	Half-life ($T_{1/2}$) ≥ 6 months
	Sediment	Half-life ($T_{1/2}$) ≥ 6 months
Bioaccumulation	Aquatic	BAF ≥ 2000 or BCF ≥ 2000 or K_{ow} ≥ 4.2 (if BAF and BCF not available)
	Terrestrial	log K_{oa} > 6 and log K_{ow} ≥ 2
	Food chain bioaccumulation potential	BMF > 1
Toxicity	**Aquatic – acute**	
	Fish	96-h LC_{50} ≤ 1 mg/L and/or
	Invertebrates	48 h EC_{50} ≤ 1 mg/L and/or
	Algae or other aquatic plants	72 or 96 h ErC_{50} ≤ 1 mg/L
	Aquatic – chronic	
	Fish	Chronic NOEC or ECx ≤ 0.1 mg/L and/or
	Invertebrates	Chronic NOEC or ECx ≤ 0.1 mg/L and/or
	Algae or other aquatic plants	Chronic NOEC or ECx ≤ 0.1 mg/L

The PBT thresholds in **Table 6-1** are similar to those derived by the EU (EC, 2003), who added additional categories of very persistent (vP) and very bioaccumulative (vB). vP compounds are considered by the EU (EC, 2003) to have a half-life in marine or freshwater of >60 days, or >180 d in marine or freshwater sediment (noting that the EU criteria for P is a half-life in freshwater of >40 days or >120 days in freshwater sediment). A substance is considered by the EU (EC, 2003) to be very bioaccumulative (vB) if it has a BCF > 5000. The different PBT thresholds are provided here as fluralaner, ivermectin and moxidectin have been classified differently for PBT, depending on the classification system.

PBT potential, as well as other chemical properties, have been used to inform the discussion on fate and behaviour of the chemicals in the environment. Relevant endpoints to the anti-parasitical compounds under consideration in this report are shown in Appendix A.

6.1 Fluralaner

Due to its slow degradation in soil and aerobic freshwater sediments, fluralaner is described as persistent/very persistent (P/vP). However, in freshwater and anaerobic freshwater sediment, fluralaner is not classified as persistent. Based on the available bioconcentration factor (BCF) data, fluralaner is also not classfied as bioaccumulative, although the reported range of octanol-water partition coefficients (log K_{ow} values) for fluralaner indicates some bioaccumulative potential.

Based on its soil adsorption coefficient (log K_{oc}), fluralaner is considered to be largely immobile in soil and therefore unlikely to partition to water during a rain event. No volatilsation data for fluralaner were found, but vapour pressure is described as very low.

Although fluralaner is reported to be insoluble in water it has been detected at a concentration of 1 ng/L in water briefly swum in by a dog treated with Bravecto® tablets (Diepens et al., 2023)[4]. This data indicates the potential for transfer of fluralaner to aquatic environments from immersion or partial immersion of treated animals. While fluralaner is stable to hydrolysis, it is subject to photolysis and will break down in both aerobic and anaerobic aquatic environments.

Given fluralaner's persistence in soil and its primary faecal excretion route (EMA, 2017b), it is feasible that off-target risks to environmental receptors could occur from exposure of fluralaner in the dung of treated animals and in soils near wombat treatment areas (Wells & Collins, 2022). Leaching to groundwater and runoff in surface water appear unlikely migration pathways. However, the data of Diepens et al. (2023) indicates the potential for treated wombats to transfer fluralaner to aquatic environments, should contact with water occur.

A summary of fluralaner's chemical properties relating to environmental fate and behaviour is provided in **Table A-12-1** in Appendix A.

6.2 Ivermectin

Ivermectin is described as persistent (P) under EU (EC, 2003) classification for soil and sediment, and the Australian P classification for soil (DCCEEW, 2022). Based on available BCF data, ivermectin is not bioaccumulative, although the reported range of octanol-water partition coefficients (log K_{ow} values) indicate some bioaccumulative potential.

Based on its water solubility and soil adsorption coefficient (log K_{oc}), ivermectin is likely to be largely immobile in soil and unlikely to partition to water during a rain event, run-off to surface water or leach to groundwater. Ivermectin has a very low vapour pressure and low Henry's Law Constant, indicating that it is unlikely to volatilise from soil or partition from water to air. However, ivermectin is subject to photolysis in soil. Degradation times in soil vary by soil type, types of microbial communities and temperature, but persistence of ivermectin in soil is expected at lower temperatures and in soils with lower pH.

Ivermectin is marginally soluble in water, but log K_{ow} data indicates that partitioning onto organic matter is likely, rather than remaining freely dissolved in the water column. Ivermectin is stable to hydrolysis at environmental pH values. Aqueous degradation and dissipation times are relatively short, but longer in sediment and whole systems. Ivermectin in aquatic systems will partition to sediment. Data on the transfer of ivermectin from treated animals to water were not found.

As with fluralaner, ivermectin's persistence in soil and primary faecal excretion route (EMA, 2017b), indicate that it is feasible that off-target risks to environmental receptors could occur from exposure of ivermectin in the dung of treated animals, noting that this would largely occur in rehabilitation and captive situations (given

[4] Volume of water in pool was 2.6 m^3 and swim time was 1.3 minutes. Time between treatment and swimming not provided.

ivermectin's use as an injectable compound delivered by veterinarians). Leaching to groundwater and run-off in surface water appear unlikely migration pathways. A summary of ivermectin's chemical properties relating to environmental fate and behaviour is provided in **Table A-12-1** in Appendix A.

6.3 Moxidectin

Moxidectin is classified as persistent (P) due to slow degradation in soils at low temperatures (12°C), although higher rates of degradation are reported at 20°C (80-140 days). Based on both BCF and octanol-water partition data, moxidectin is also bioaccumulative (B).

Based on its soil adsorption coefficient, and as with both fluralaner and ivermectin, moxidectin will be largely immobile in soil and therefore unlikely to partition to water during a rain event, run-off to surface water or leach to groundwater. It also has low water solubility, indicating that moxidectin in water will tend to partition out of the water column to organic matter and sediment. Animal wash-off trials indicate that less than 1% of the applied dose was found to wash off treated cattle, when rainfall occurred within 30 minutes of application, noting that 1%, whilst low, may still be a significant mass for exposed soil and aquatic organisms.

Moxidectin is subject to photolysis and breaks down fairly rapidly in water. Aqueous degradation times in sediments and sediment + water systems were not found in the preparation of this report. In soil, degradation times vary with temperature and soil type, but degradation times (DT_{50} values) are of the order of two to ten months.

7 Predicted Environmental Concentrations

7.1 Approach

An estimation of the concentration of each anti-parasitical chemical in both soil and dung (faeces) was made. The approach adopted followed the European Medicines Agency Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38 (the Guideline) (CVMP, 2016). The purpose of the Guideline is to provide specific technical guidance on environmental impact assessment of veterinary products, in particular risks to terrestrial and aquatic environments through the application of manure, dung and urine.

The Guideline enables derivation of predicted environmental concentrations (PECs) for various environmental compartments (e.g., soil and dung), which can be refined through the application of additional factors such as metabolism of the veterinary chemical, it's excretion pattern from treated animals and how the active chemical degrades in soil. Although intended for livestock in both intensively-reared and pasture situations, the Guideline provides a benchmark approach to estimate PECs, and then compare these with toxicity data for environmental organisms. It is noted that the PECs are estimates of exposure in spatially defined areas (such as feedlots and pasture-grazing areas), rather than a more diffuse estimate of risk to the environment, or an estimate in a localised hot-spot (such as the entrance to a wombat burrow).

The Guideline considers that the terrestrial environment may be exposed to veterinary medicines via (1) direct excretion of dung and urine; (2) loss from animals treated topically; and (3) spreading of contaminated slurry and/or sludge. Of these terrestrial exposure pathways, only (1) and (2) are relevant for the chemical use patterns considered in this report (see Section 2). For aquatic environments, the Guideline considers five exposure pathways of which only (1) leaching, run-off and drainage from manured land; and (2) direct spillage and/or feed spillage are the only relevant pathways for the chemical use patterns considered herein.

To support the assessment of potential environmental effects associated with the use of Bravecto®, ivermectin and Cydectin® in treating sarcoptic mange in free-ranging wombats, preliminary PECs of fluralaner, ivermectin and moxidectin in soil and wombat dung have been previously calculated (L. Burgess, *pers. comm*.). This report draws from the preliminary PEC data and discusses these data in the context of the environmental effects information.

7.1.1 PEC Soil

PEC soil values can be calculated as both $PEC_{soil\ initial}$ and $PEC_{soil\ refined}$, with the former a conservative estimate of soil concentration, without considerations of metabolism, degradation, etc. For the purposes of this report, only $PEC_{soil\ initial}$ has been estimated, as the calculation has only limited relevance for the exposure scenario considered herein (targeted treatment of individual wild wombats), as opposed to treatment of multiple livestock within a confined area.

$PEC_{soil\ initial}$ is calculated according to the following:

$$PEC_{soil\ initial} = \left(\frac{D\ x\ A_d\ x\ BW\ x\ SD\ x\ F_h}{BD\ x\ CF\ x\ PD}\right)$$

Equation 1

Where:

$PEC_{soil\ initial}$	=	Predicted environmental concentration in soil (mg/kg)
D	=	Daily dose of the active ingredient ($mg.kg_{bw}.d$); varies by veterinary chemical
Ad	=	Number of days of treatment (d); assume 1
BW	=	Animal body weight (kg); assume 25 kg (expected range 20-35 kg)
SD	=	Stocking density (ha^{-1}); assume 0.25 / ha (Evans, 2008)
F_h	=	Fraction of population treated (value between 0 and 1); assume 1 (all wombats treated/ha)
BD	=	Bulk density of dry soil (kg/m^3). Default value 1,500 kg/m^3, but a lower value of 954 kg/m^3 applied for wombat areas
CF	=	Conversion factor m^2 / ha (10,000 m^2/ha)
PD	=	Penetration depth into soil (m); 0.05 m (Kulik & Belknap, 2015)

As can be seen from Equation 1, the PEC_{soil} calculation averages the applied dose over a mass of soil comprising one hectare in area and five centimetres in depth. In field situations associated with treatment of free-ranging wombats, transfer of the veterinary chemicals to soil will be highly heterogeneous, with "hot-spots" of contamination near treatment locations (from spillages, run-off and shake-off), and in areas where treated wombats defecate, but little to no detectable concentrations over large areas of surrounding soils. Hence this approach likely results in under-estimations of localised soil concentrations, but also over-estimations of soil concentrations in areas away from treatment locations and wombat toileting areas.

7.1.2 PEC Dung

PEC values can also be calculated for dung (PEC_{dung}) for applied ecto-parasitides. Preferably these are calculated from absorption-distribution-metabolism-excretion (ADME) studies. When this information is not available, a worst-case maximim is calculated using the equation below:

$$PEC_{dung} = \frac{D\ x\ BW\ x\ F_{dh}}{M_{dung}}$$

Equation 2

Where:

PEC_{dung}	=	Predicted environmental concentration in dung (mg/kg_{wwt})
D	=	Daily dose of the active ingredient ($mg.kg_{bw}.d$); varies by veterinary chemical
BW	=	Animal body weight (kg); assume 25 kg
Fdh	=	The highest fraction of the dose excreted in dung in 1 day
M_{dung}	=	Mass of dung produced in one day (kg/d, wet weight)

PEC_{dung} was calculated as both *initial* and *refined* concentrations, where the initial calculation set Fdh to a value of one (all of the applied dose excreted unchanged in one day). The refined PEC_{dung} revised the Fdh to an estimated daily excretion rate of the applied dose.

Values for Fdh (the highest fraction of the dose excreted in dung in one day) were estimated from the literature. For fluralaner, Wilkinson et al. (2021) indicate a half-life of 40 days at lower doses and 165 days at higher doses (section 4.3). Noting that elimination is not linear, an approximate time for elimination of lower doses is estimated as 80 days, and daily elimination is estimated as 0.0125/d. For higher doses, the approximate time of elimination is 330 days, with daily elimination of 0.003/d. For ivermectin, Vercruysse and Rew (2002) reported a half-life in livestock of 127.2 hours (5.3 days) when applied as a pour-on (section 4.3). Assuming linear elimination, this half-life indicates an elimination period of 10.6 days (254.4 hours), with daily elimination of 0.094/d. For moxidectin, an elimination half-life of five days in wombat has been reported by Death et al. (2011). Again, assuming linear elimination, an elimination period of 10 days is calculated, with daily elimination of 0.1/d[5].

In terms of mass of dung, wombats are reported to produce 80-100 scats per day (Triggs, 2009), with an average weight of 9.1 g (Yang et al., 2021). These data result in a total mass of dung produced in one day of approximately 0.82 kg (wet weight). Yang et al. (2021) indicate that the water content of wombat dung in the distal colon is approximately 53%. As some toxicological data is reported in dry weight, wet weight concentrations were converted to dry weight, as per the following:

$$PEC_{dung\ dwt} = PEC_{dung\ wwt} x \frac{1}{(1 - WC)}$$

Equation 3

Where:

PEC_{dung} dwt	=	Predicted environmental concentration in dung (mg/kg dry weigtht)
PEC_{dung} dwt	=	Predicted environmental concentration in dung (mg/kg wet weigtht) – from Equation 2
WC	=	Water content, unitless, 0.53 (Yang et al., 2021)

The following sections provide the calculations for *PEC-soil initial* and *PEC-dung* (initial and refined) for each of fluralaner, ivermectin and moxidectin.

[5] It is noted that Doran et al. (2024) reported that 90% clearance of moxidectin in a single wombat took 28 days when administered 100 mL of Cydectin®

7.2 Estimated PECs for Soil and Dung

The estimated PECs for soil (initial) and dung (initial and refined) are shown in **Table 7-1**. As indicated earlier, $PEC_{dung\ initial}$ concentrations are calculated assuming that the entire administered dose is excreted in the dung in one day. $PEC_{dung\ refined}$ concentrations are calculated assuming that daily elimination of the dose occurs evenly over a duration equal to twice the half-life. $PEC_{dung\ initial}$ concentrations are conservative "first-pass" estimations that over-estimate concentrations of the chemicals in dung. $PEC_{dung\ refined}$ concentrations are more likely to approximate daily dung concentrations during the elimination period.

Table 7-1. Summary of estimated PECs for Soil and Dung

	Fluralaner		Ivermectin		Moxidectin		
Dose ($mg/kg_{bw}.d$)	25	56	0.2	0.4	4	20	40
$PEC_{soil\ initial}$ (mg/kg_{soil})	**0.000327**	**0.0007**	**0.000003**	**0.0000052**	**0.0000524**	**0.00026**	**0.00052**
Highest fraction excreted - initial	1	1	1	1	1	1	1
$PEC_{dung\ initial}$ (mg/kg_{dung} ww)	762	1707	6.10	12.2	122	610	1220
$PEC_{dung\ initial}$ (mg/kg_{dung} dw)	1622	3633	13	26	259	1297	2595
Elimination half-life (days)	40	165	5.3			5	
Highest fraction excreted – refined (per day)	0.0125	0.003	0.094	0.094	0.1	0.1	0.1
$PEC_{dung\ refined}$ (mg/kg_{dung} ww)	9.53	5.17	0.575	1.15	12.2	61	122
$PEC_{dung\ refined}$ (mg/kg_{dung} dw)	**20.3**	**11.1**	**1.22**	**2.45**	**25.9**	**130**	**259**
Mass of chemical per scat* (mg / scat, ww)	**0.087**	**0.047**	**0.005**	**0.01**	**0.111**	**0.555**	**1.11**

*Assumed scat weight of 9.1 g; ww = wet weight; dw = dry weight

As can be seen in **Table 7-1**, administration of fluralaner at higher doses appears to result in lower concentrations in dung (and per scat) than lower doses. This is because of the proportionally longer elimination time reported by Wilkinson et al. (2021) for a higher dose of fluralaner, which results in a lower daily concentration in scat.

The PECs for soil and dung are discussed further in section 8.

8 Effects on Non-Target Species

The following sections discuss the effects (toxicity) data, where available, to terrestrial vertebrates (birds and mammals), aquatic species (fish, aquatic invertebrates and algae), bees and non-target arthropods, dung organisms and earthworms, terrestrial plants and soil microorganisms. This approach is generally consistent with that of the Australian Pesticides and Veterinary Medicines Authority, in their environmental assessments of new pesticides and pesticide formulations.

A summary of the toxicity endpoints is provided in Appendix B.

8.1 Effects on terrestrial vertebrates

Chronic toxicity data to birds – through dietary and reproductive studies – were not found for any of the three anti-parasitical chemicals under consideration in this report. Acute toxicity data to birds, and acute and chronic toxicity to mammals is discussed in sections 8.1.1 to 8.1.3 below.

8.1.1 Fluralaner

Fluralaner has relatively low acute and chronic toxicity to mammals via multiple exposure routes, with an acute LD_{50} to rats of >2000 mg/kg_{bw} and chronic NOEC values 1 to 100 mg/kg_{bw} (refer to Appendix B). In addition, Wells & Collins (2022) state that Bravecto® administration to African pygmy hedgehog at 15 mg/kg_{bw} and rabbits and wombats at 25 mg/kg_{bw}, did not result in measurable adverse effects. Similarly, Van Wick and Hashem (2019) reported that a single oral dose of fluralaner at 44.16 mg/kg to an American black bear did not appear to result in measurable adverse effects.

Limited data were available for birds, although EMA (2017b) report that treatment of layer hens at up to five times the recommended therapeutic dose (RTD) and for three times the recommended duration of treatment had no negative effect on egg production. Similarly, no adverse effects on reproductive performance were reported when breeding chickens were treated at three-times the RTD for two-times the recommended duration of treatments (EMA, 2017b).

Detectable concentrations of fluralaner in hair founds in birds' nests has been found in the Netherlands (Diepens et al., 2023), and Guldemond et al. (2019)[6] found fluralaner (and other pesticides) at detectable concentrations in dead juvenile birds, although the latter study noted that an assessment of pesticide levels in live juvenile birds was not undertaken. These studies provide evidence that animal husbandry compounds can be transferred to wild avian fauna following livestock treatment, although effect levels in wild birds are not currently known.

8.1.2 Ivermectin

Ivermectin is considered to have relatively low toxicity to birds, chickens, ducks and mammals (APVMA, 2006). Clinical signs of toxicity to domestic cats and dogs are at least an order of magnitude higher than therapeutic dosages in animals with normal responses to ivermectin exposure (noting a particular gene deletion can cause sensitivity in some dog breeds) (Merola & Eubig, 2018). Given the mode of action of macrocyclic lactones, and method of application to captive wombats (injection rather than pour-on), toxicity to wild avian and mammalian species is considered unlikely.

8.1.3 Moxidectin

Moxidectin displays low toxicity to mammals in both short and long-term studies, with acute LD_{50} values around 40 – 100 mg/kg_{bw} and chronic LD_{50} values around 0.4 – 5 mg/kg_{bw}. As moxidectin has been approved for use in many livestock animals, including dairy and beef cattle, horses, sheep and red deer, as well as pet animals such as dogs and cats (Prichard & Geary, 2019), there appears to be broad tolerance of treatment doses of moxidectin across mammalian species. Overall, the toxicity of moxidectin to mammals appears to be lower than that of ivermectin, but similar to fluralaner (at least in long-term toxicity studies).

The acute toxicity of moxidectin to birds is also low, with LD_{50} values >200 mg/kg_{bw}. A comparison with fluralaner and ivermectin could not be made as quantitative toxicity endpoints were not available for these

[6] Original reference: Guldemond A, Gommer R, Leenderste P, van Oers K (2019) Koolmezensterfte en buxusmotbestrijding. Reference is in Dutch and not found during this review.

chemicals. FDA (2003) report that moxidectin is unlikely to have impacts on hair-eating birds (via ingestion of hair from topically-treated animals), based on estimations of the concentrations of moxidectin in the fur of treated cattle, combined with hair intake of magpies.

8.2 Effects on aquatic species

No toxicity data on aquatic plants were available for any of the three anti-parasitical chemicals under consideration in this report. Toxicity data to fish, aquatic invertebrates and algae are discussed further below.

8.2.1 Fluralaner

Fluralaner appears to have low acute toxicity to fish. For zebrafish (*Danio rerio*) an LD_{50} was reported at greater than 10 mg/L, which is above the limit of solubility, indicating that concentrations of fluralaner in the environment are unlikely to reach acute toxicity thresholds for fish. In rainbow trout (*Oncorhynchus mykiss*), no acute toxicity was reported at the limit of solubility (>0.049 mg/L). Chronic toxicity data are limited, with one study finding that the NOEC in fathead minnow (*Pimaphales promelas*) is at or greater than the limit of solubility (≥0.049 mg/L).

Acute toxicity also appears to be low to aquatic invertebrates, with an EC_{50} of >0.015 mg/L to *Daphnia magna* reported. However, chronic toxicity to *Daphnia magna* appears to be high, with two studies reporting NOEC values < 0.1 µg/L. On this basis, fluralaner is classified as toxic (T) as per PBT classification. No data was available on the effects of fluralaner on sediment-dwelling aquatic invertebrates or aquatic plants. For algae a single study reported a NOEC >0.08 mg/L, at or above the limit of solubility.

8.2.2 Ivermectin

Ivermectin is classified toxic (T) under PBT classification for aquatic organisms, given its high toxicity to fish and very highly acute toxicity to aquatic invertebrates, with LC/EC_{50} values less than 50 ng/L to *Daphnia magna*, *Gammarus sp*. and *Neomysis integer* (amphipod crustaceans). Ivermectin also displays a high level of chronic toxicity to multiple groups of aquatic organisms: aquatic invertebrates (*Daphnia magna*; NOEC values less than 1 ng/L); sediment dwellers (*Chironomus riparius*, and *Lumbriculs variegatus*) and algae (*Raphidocelis subcapitata*, formerly known as *Pseudokirchneriella subcapitata*).

8.2.3 Moxidectin

As with ivermectin, moxidectin is highly acutely toxic to fish, with LC_{50} values <1 µg/L. On this basis, moxidectin is classified as toxic (T) under PBT classification for aquatic organisms. Moxidectin also displays a high level of chronic toxicity to fish (NOEC value of 3.2 ng/L).

Moxidectin is highly acutely toxic to aquatic invertebrates, with an EC_{50} value of 0.05 µg/L to *Daphnia magna*. A high level of chronic toxicity to aquatic invertebrates is also shown (NOEC 0.003 µg/L).

With respect to sediment dwellers, no acute toxicity data were found in the preparation of this report, but a NOEC for chronic effects on *Chironomus riparius* was reported as 235 µg/kg. Moxidectin is toxic to algae, with EC_{50} values less than 1 mg/L.

Overall, moxidectin displays both acute and chronic toxicity to a range of aquatic receptors.

8.3 Effects on bees and non-target arthropods

Potential exposure routes of bees to anti-parasitical chemicals used in animal husbandry and in treatment of wombats are not immediately obvious. However, UNAF (2018) proposed that bees may forage for water from puddles filled with decomposing organic matter and dung, as bees have a preference for water sources rich in sodium, ammonium and magnesium. UNAF (2018) also stated that bees can drink directly from the wet fleeces of treated animals, and from the fluids that flow from them. Another potential exposure route of animal husbandry chemicals to bees is the generation of dust from cattle farms, which can contaminate wildflowers. UNAF (2018) report that moxidectin has been detected in wildflowers and in wetlands around large Texas livestock farms.

These potential exposure routes to bees are of greater concern for bees near feedlots and intensively grazed areas, rather than the individual treatment of free-ranging or captive wombats. For completeness, the potential for bee exposure is included herein, and to provide context with the discussion on bee toxicity that follows.

8.3.1 Fluralaner

No data were reported on the direct contact effects of fluralaner on bees or non-target arthropods from soil exposure. Topical studies were available on a number of insects, including red flour beetle (*Tribolium castaneum*), fall armyworm (*Spodoptera frugiperda*) and the Asiatic rice borer (*Chilo suppressalis*). Oral studies were available for the fruit fly (*Drosophilia melanogaster*), yellow-fever mosquito (*Aedes aegypti*) and the fall armyworm. The nature of these studies (topical and ingestion) does not readily support an assessment of toxicity by the expected exposure routes considered herein for insects that do not rely on dung as part of their lifecycle. However, contact toxicity of fluralaner was typically reported around 0.003 – 0.065 µg/insect (refer Appendix B). As a single wombat scat is estimated to contain 47 – 87 µg, fluralaner in wombat dung is expected to be acutely toxic to insects that come in direct contact with dung.

8.3.2 Ivermectin

Two studies indicate that ivermectin is toxic to bees, via both oral and contact exposure routes (refer to data in Appendix B). There is also some evidence that ivermectin causes sublethal effects in bees, by reduction of long-term olfactory memory (UNAF 2018).

Based on the PEC calculations in section 7.1.2, dung from wombats is expected to contain approximately 600 µg/kg to 1,200 µg/kg ww ivermectin, with a mass in individual scats of approximately 5 to 10 µg / scat ww. These values are above the contact toxicity LD_{50} of 1.32 µg/bee (refer Appendix B), indicating that ivermectin in wombat dung could be toxic to bees, should contact occur.

There are a considerable number of toxicity datapoints for exposure of springtails (collembolans) to ivermectin, with NOEC values reported at 0.3 – 0.4 mg/kg soil. These concentrations are well above the PEC_{soil} values calculated in section 7.1.1, but as previously described, the PEC_{soil} values are average soil concentrations over a hectare (from a 0.25 wombat//hectare stocking density) and are therefore of limited value in estimating soil toxicity from the anticipated localised “hot-spot” contamination associated with spillages, run-off, shake-off and leaching from dung. By way of comparison, estimated concentrations of ivermectin in wombat dung are approximately 1.2 – 2.5 mg/kg (dw, refer **Table 7-1**), above the NOEC values for springtails and indicating potential toxicity in areas where ivermectin in decomposing dung transfers to soil.

8.3.3 Moxidectin

Moxidectin is toxic to bees, via both oral and contact exposure routes (refer Appendix B). The data indicate greater toxicity of moxidectin to bees than ivermectin.

Based on the PEC calculations in section 7.1.2, dung from wombats is expected to contain approximately 12,000 to 122,000 µg/kg ww moxidectin, with a mass in individual scats of approximately 100 to 1000 µg / scat ww, depending on the applied dose. These values are well above the contact toxicity LD_{50} of 0.025 µg/bee (refer Appendix B), indicating that moxidectin in wombat dung could be toxic to bees, should contact occur.

No data were found on the toxicity of moxidectin to springtails in soil.

8.4 Effects on dung organisms and earthworms

8.4.1 Fluralaner

A single toxicity endpoint of fluralaner was available for dung organisms. This involved topical application of fluralaner to horn fly (*Haematobia irritans*), with a reported LD_{50} of 2.9 ng/insect. The estimated fluralaner concentration in a single wombat scat is 47 to 87 µg/scat(refer **Table 7-1**). On this basis (and assuming the application rates and excretion rates discussed in section 7.1.2), scat from fluralaner treated wombats is expected to be acutely toxic to horn fly and potentially other dung organisms.

No data were found on the effects of fluralaner to earthworms.

8.4.2 Ivermectin

Multiple studies were available on the toxicity of ivermectin to dung fauna, as summarised by de Souza & Guimaraes (2022). The dung from calves (injected) with a dose of ivermectin of 0.2 mg/kg_{bw} has been shown to be acutely toxic to newly emerged dung beetles *Copris hispanus* adults that fed on dung voided 2-16 days after injection. In the same study, oviposition rate was reduced and immature survival was zero when dung beetles were fed with day three dung. In addition, other studies have shown that at an injected dose of 0.2 mg/kg_{bw}, in cattle, emergence of adult *Euoniticellus intermedius* and *Onthophagus gazella* was reduced for one and two weeks

Ivermectin has a high level of toxicity to house flies (*Musca autumnalis*) and dung fly (*Scathophaga stercoraria*), with EC_{50} values less than 0.005 mg/kg ww (refer to Appendix B). Based on the PEC calculations in section 7.1.2, dung from wombats is expected to contain approximately 0.6 mg/kg to 1.2 mg/kg ww ivermectin. Noting that the PEC_{dung} calculations are conservative, these concentrations are at least an order of magnitude higher than multiple EC/LC_{50} and NOEC values for dung fauna. Based on this comparison, dung from wombats treated with ivermectin is likely to be acutely toxic to dung fauna larvae and adults.

Ivermectin is moderately toxic to earthworms, with NOEC values reported at 2.5 – 12 mg/kg for effects on reproduction and biomass. The reported NOEC values are higher than estimated concentrations of ivermectin in dung (approximately 1.2 to 2.5 mg/kg dw, refer **Table 7-1**), indicating a low likelihood of impacts to earthworms at the treatment doses.

8.4.3 Moxidectin

The toxicity of moxidectin to dung fauna appears to be lower than that of ivermectin. The available EC_{50} and NOEC values are at least an of magnitude higher for moxidectin than ivermectin (indicating lower toxicity; refer to Appendix B) although direct like-for-like comparisons were only available for *Aphodius constans*. NRA (1998) state that moxidectin residues in cattle dung are considered non-toxic to egg-laying adults and developing larvae. In addition, under European field conditions, colonisation and larval development of native Coleoptera in dung pats were unaffected by moxidectin residues (NRA, 1998).

Similarly, Lumaret et al. (2012) report that moxidectin administered topically or as an injectable treatment 1 to 42 days previously had no effect on reproductive success of the dung beetles *Euoniticellus intermedius* and *Digitonthophagus gazella*. Furthermore, moxidectin added directly to fresh cattle dung also had no effect on the fecundity of *D. gazella* at concentrations of 4 to 512 µg/kg, although larval survival was reduced at concentrations of 256 to 512 µg/kg. In other experiments, dung from cattle topically treated with moxidectin had no detectable effects on the dung beetle *Onthophagus taurus* (cattle treatment 3 to 70 days prior) or the dung beetle *Caccobius jessoensis* (treatment 1 to 28 days prior).

However, based on the PEC calculations in section 7.1.2, dung from wombats is expected to contain approximately 12 mg/kg to 122 mg/kg ww moxidectin (depending on the applied dose). These concentrations are at least an order of magnitude higher than multiple EC_{50} values for dung fauna (refer to Appendix B), and also higher than the concentrations of moxidectin reported in cattle dung in Lumaret et al. (2012). Noting that the PEC_{dung} calculations are likely to be conservative, this comparison indicates that dung from wombats treated with moxidectin (especially at high treatment concentrations) could be acutely toxic to dung fauna.

Moxidectin toxicity to earthworms is similar to or higher than that of ivermectin (refer to Appendix B), with a NOEC for reproduction of 0.84 mg/kg to *Eisenia foetida*. This concentration in soil is significantly higher than the PEC_{soil} values estimated in section 7.1.1, noting as previously that these are “average” PEC_{soil} values and unlikely to be representative of local “hot-spot” contamination. Earthworms exposed to dung from wombats at the two higher treatment doses considered is expected to be toxic (LC_{50} 37.2 mg/kg, PEC_{dung} 26 – 259 mg/kg dw, refer to **Table 7-1** and Appendix B).

8.5 Effects on terrestrial plants

Plant species can be impacted by anti-parasitical chemicals via direct toxicity, but also indirect toxicity. The latter is related to their impact on dung organisms, which may impact plant regeneration via reduced breakdown of animal dung. The impacts of animal husbandry chemicals on plants are not fully understood, and Eichberg et al. (2016) state that studies are necessary to test both active ingredients and formulations on plants.

8.5.1 Fluralaner

No data on the effects of fluralaner on terrestrial plants were found.

8.5.2 Ivermectin

De Souza & Guimaraes (2022) state that early tests involving avermectins on plants indicated that plant leaves were not able to absorb these chemicals, and that ivermectin is rapidly degraded in cotton leaves, probably through photodegradation. However, ivermectin is reported to have some toxicity to plants, with a NOEC of 0.56 mg/kg for vegetative vigour reported (Lumaret et a. 2012, refer Appendix B). Ivermectin has been reported in high concentrations in plants growing close to dung pats containing ivermectin, suggesting movement of the chemical from dung to both underlying soil and plants (de Souza & Guimaraes, 2022). Observations of plants exposed to ivermectin include a decrease in root growth at a concentration of 50 nM (43.8 µg/L) (de Souza & Guimaraes, 2022).

Vokral et al. (2023) summarised the effects of a range of veterinary chemicals, including ivermectin, on plants. Reported effects included changes in uptake, biotransformation, antioxidative enzyme activities and omics (transcriptomics and proteomics) as low as 42 µg/kg dry soil.

8.5.3 Moxidectin

Although no toxicity endpoints were found for plants exposed to moxidectin, De Souza & Guimaraes (2022) state that moxidectin can negatively affect plant species in temperate grasslands. Sheep dung containing moxidectin significantly reduced the germination of *Centaurea acea, Galium verum,* and *Plantago lanceolata* species, and the commercial formulation Cydectin® also demonstrated phytotoxic action (de Souza & Guimaraes, 2022). Similarly, the number of seedlings emerging out of the dung of sheep treated with Cydectin (0.1% of moxidectin) was significantly lowered by almost two-thirds, compared to seedling emergence from the dung of untreated sheep (Eichberg et al., 2016). This finding is also confirmed by the work of Laber et al. (2023), who found that moxidectin impacted the quantity and timing of seedlings that emerged from seeds ingested by sheep and released embedded in dung. Laber et al. (2023) also reported both positive and negative effects on seedling emergence, depending on the dose applied to the treated sheep.

Conversely, FDA (2003) report that moxidectin is not phytotoxic to a wide variety of plants when applied either pre-emergence to soil, or post-emergence to weeds, at a rate of 4 kg/ha, which is well above PEC_{dung} concentrations. The FDA (2003) report predates those of de Souza & Guimaraes (2022), Eichberg et al. (2016) and Laber et al. (2023) and as such, greater reliance is placed on the latter studies, which indicate the potential phytotoxicity of moxidectin.

8.6 Effects on soil microorganisms

8.6.1 Fluralaner

No data were found on the effects of fluralaner on soil microorganisms.

8.6.2 Ivermectin

Avermectins are reported to not possess significant antibacterial activity except at extremely high concentrations (Halley et al., 1993). Ivermectin in dung of steers at 30 µg/kg did not affect soil nitrification or respiration (Halley et al., 1993).

8.6.3 Moxidectin

No data were reported on the effects of moxidectin on soil microorganisms.

8.7 Summary of Effects on Non-Target Species

A summary of the effects data is provided in **Table 8-1**.

Table 8-1. Summary of toxicity data by receptor group

Receptor group	Fluralaner	Ivermectin	Moxidectin
Birds and mammals	Low toxicity to mammals Probable low toxicity to birds, although data are limited Evidence of fluralaner transfer to nests and juveniles of wild birds	Low toxicity to mammals Probable low toxicity to birds	Low toxicity to mammals Low toxicity to birds
Aquatic organisms	Low toxicity to fish Chronic toxicity to aquatic invertebrates Low toxicity to algae No data for sediment dwellers	Acutely toxic to fish Acute and chronic toxicity to aquatic invertebrates Toxic to algae Chronic toxicity to sediment dwellers	Acute and chronic toxicity to fish Acute and chronic toxicity to aquatic invertebrates Toxic to algae Chronic toxicity to sediment dwellers
Bees and non-target arthropods	No data for bees Potential toxicity to non-target arthropods via contact	Toxic to bees Toxic to springtails	Toxic to bees No data on springtails
Dung organisms and earthworms	Toxic to dung organisms No data for earthworms	Toxic to dung organisms Moderately toxic to earthworms	Potential toxicity to dung organisms Moderately toxic to earthworms
Terrestrial plants	No data	Potential toxicity	Potential toxicity
Soil microorganisms	No data	Toxicity not observed	No data

Based on the available data, the main organisms potentially affected by the anti-parasitical compounds are aquatic organisms, particularly aquatic invertebrates, and dung organisms. This result is not unexpected, given the mode of action of these chemicals and metabolism/excretion patterns. There are additional receptors such as birds, bees, soil microorganisms and terrestrial plants, for which the data are insufficient to fully understand potential impacts, but for which there are indications of potential transfer and/or exposure.

With respect to the exposure of aquatic organisms, the properties of fluralaner, ivermectin and moxidectin are such that run-off and wash-off to aquatic systems (creeks, ponds etc) is unlikely, as they tend to bind to, and be largely immobile in, soil. However, concentrations that are toxic to aquatic life are extremely low, indicating that leaching or run-off of only a very small mass to a waterbody may be sufficient to cause toxicity to aquatic

organisms. By way of example, the estimated spillage rate is 20% of an intended mid-level dose volume of Cydectin®. The total intended dose of for a 25 kg wombat is 500 mL, of which 100 mL (or 500 mg moxidectin) is anticipated to be spilt over the five week treatment period (refer to **Table 3-2**). If only 1% of this spilt mass (5 mg) were to enter a waterbody of 100 m^3 (10 m x 10 m x 1 m deep; 100,000 L), this would result in a concentration greater than the lowest adverse effect level for aquatic invertebrates (0.000025 mg/L), indicating the potential for impacts on aquatic organisms. It is noted that moxidectin is subject to photolysis and may breakdown fairly rapidly in areas exposed to sunlight, so the accumulated concentration in soil over multiple spillage events may not be achieved.

Alternatively, should a Cydectin® treated wombat enter a waterbody and 1% of the applied dose[7] run-off or wash-off into the same waterbody, the same outcome could be expected.

Similar calculations can be made for fluralaner, with fluralaner's persistence in soil indicating that concentrations could build up in soil over time, increasing the potential for run-off to aquatic systems, prior to degradation.

Greater spilt volumes and/or treatment concentrations, as well as additional wombats undergoing treatment in the same catchment area have the potential to increase receiving waterbody concentrations.

With respect to dung organisms, the available data for moxidectin indicates lower toxicity to dung fauna compared with ivermectin, although direct faunal comparisons (same species, same endpoints) were only available for one species (*Aphodius constans*). Insufficient data were available for fluralaner to compare with moxidectin in relation to toxicity to dung organisms. The greatest reduction in impacts to dung organisms associated with free-ranging wombats is likely to occur through use of the minimum effective dose, rather than changes to application method.

There remains the potential for other off-target receptor risks (birds, other invertebrates, soil microorganisms and plants) associated with all three chemicals. Currently, the data are insufficient to assess the likelihood of effects on these receptor groups or to differentiate between the three chemicals with respect to potential risks. It is to be expected, however, that the dung of treated wombats is likely to have a level of toxicity to many arthropods (beneficial or otherwise) until concentrations degrade to those below effect levels. Data on degradation times in dung (rather than soil) were not found in the preparation of this report, but soil degradation times are likely to be a reasonable approximation, and DT_{50} values for soil are of the order of months (moxidectin) to years (fluralaner, refer Appendix B). There may also be impacts to vegetation in the areas where wombats frequently defecate, and at treatment locations. With respect to birds, some transfer of the chemicals to nests may occur via adult birds using the hair from treated wombats to line nests, although the impacts to birds associated with this potential transfer are currently not understood and unlikely to be significant where wombat treatment densities are low. Given the lower likelihood of bird exposure, it is considered that the focus for potential environmental risks associated with the treatment of free-ranging wombats should be on soil, dung and aquatic organisms in treatment areas.

8.8 Comparison between Treatment Chemicals

A comparison of key aspects of the anti-parasitical chemicals is provided below in Table 8-2, with comparable endpoints listed for key receptor groups, where possible.

[7] 1% of 400 mg (refer to **Table 3-2**), assuming 20% spilt, equates to 4 mg

Table 8-2. Summary of Key Aspects of the Anti-parasitical Chemicals

Aspect	Fluralaner	Ivermectin	Moxidectin
Metabolism	Not extensively metabolised and largely excreted unchanged. Can accumulate with frequent administration.	Not extensively metabolised and excreted largely unchanged	Potential for a lower proportion of parent compound excreted in wombat dung. Further data needed
Plasma half-life (days)	40 days	1.5 – 2.7	5 days
Breakdown in soils	Very slow breakdown (DT_{50} values >400 days)	DT_{50} values 16 – 458 days, soil dependent	DT_{50} values 78 – 297 days
Breakdown in water	DT_{50} values ~ 3.5 days	DT_{50} values <3 days (dissipation)	Half-life in water through photodegradation 6.8 hours
Breakdown in sediment / whole system	DT_{50} values 8.5 – 86 days (sediment)	DT_{50} 15 - 127 days 130 days (whole system)	No data
Aquatic toxicity	Highly toxic to one or more groups of aquatic organisms	Highly toxic to one or more groups of aquatic organisms	Highly toxic to one or more groups of aquatic organisms
Dung fauna	Data indicate relatively high toxicity (not confirmed)	Highly toxic	Toxic
PBT	Persistent / very persistent Not bioaccumulative Toxic	Persistent Not bioaccumulative Toxic	Persistent Bioaccumulative Toxic

Based on a chemical-by-chemical comparison, moxidectin, with its shorter degradation time in soils and water, and comparatively lower toxicity to dung fauna would appear to present a lesser risk to off-target environmental receptors. This is the case even though greater volume losses of Cydectin® to the environment are expected compared with Bravecto®, because the concentration of fluralaner in Bravecto® is much higher than the concentration of moxidectin in Cydectin®. There is also some data to indicate that moxidectin is associated with a greater proportion of lower activity metabolites in dung, although this is currently unknown for wombats.

9 Uncertainties and Data Gaps

The main uncertainties and data gaps associated with the environmental risk profile of fluralaner, ivermectin and moxidectin relate to the following:

- The proportion of active moxidectin, fluralaner and ivermectin in the dung of wombats;
- Dissipation and degradation of moxidectin in aquatic systems;
- The effects of fluralaner and moxidectin on soil microorganisms; and
- The effects of fluralaner on terrestrial plants.

Environmental monitoring studies of residual concentrations of the anti-parasitical chemicals in dung, soil, plants and water in or near to treatment areas of free-ranging wombats would provide information beneficial to

understanding how the treatment chemicals behave in the environment, and whether they transfer to other environmental matrices (such as plants) or migrate away from treatment areas and wombat toileting areas. The results of these studies could then further inform treatment protocols and restrictions associated with sarcoptic mange in free-ranging wombats.

10 Summary and Key Recommendations

10.1 Summary

Arcadis was engaged by WHA to undertake a review of the environmental effects of the off-label use of the anti-parasitical chemicals fluralaner, ivermectin and moxidectin in the treatment of sarcoptic mange in wombats.

The treatment of sarcoptic mange in free-ranging wombats can involve the topical application of veterinary products Bravecto® (containing fluralaner) and Cydectin® (containing moxidectin), which are registered for use in domestic animals such as dogs and cattle. Ivermectin is generally used by injection to captive wombats or those under rehabilitation. During and following application of Bravecto® and Cydectin®, some of the active chemical(s) may enter the environment through spills as well as run-off and shake-off from the wombat and/or through residues in urine/dung and subsequent leaching into soil and water.

The three chemicals under consideration generally report low toxicity to birds and mammals, but high toxicity to aquatic organisms (fish, aquatic invertebrates and/or sediment dwellers) and dung fauna. Whilst the properties of the chemicals under consideration indicate a low likelihood of leaching from soil and dung, aquatic toxicity can occur at ng/L concentrations (i.e. extremely low), indicating that minimising the potential transfer of the chemicals to waterways is a key consideration. There are gaps in the understanding of the migration of these chemicals in the terrestrial environment and the potential uptake by, and effects on, plants, as well as potential effects on bees and soil microorganisms. There is some evidence to indicate transfer to plants growing adjacent to areas where animals have been treated.

Based on the information available for review, moxidectin, with its shorter degradation time in soils and water, and comparatively lower toxicity to dung fauna would appear to present a lesser risk to off-target environmental receptors. This is the case even though greater volume losses of Cydectin® to the environment are expected compared with Bravecto®, because the concentration of fluralaner in Bravecto® is much higher than the concentration of moxidectin in Cydectin®. There is also some data to indicate that moxidectin is associated with a greater proportion of lower activity metabolites in dung, although this is currently unknown for wombats.

Due to the excretion of un-metabolised treatment chemicals in wombat dung, the treatment of sarcoptic mange in wombats will inevitably result in the transfer of some of chemicals to the environment, regardless of which chemicals are used, or the treatment method. A key factor in mitigating environmental risk is associated with the applied dose and the application method; that is, adoption of the minimum effective dose that reduces the likelihood of spillage, as well as run off and shake-off from treated wombats.

With respect to aquatic receptors, risk mitigations could include avoiding the use of the chemicals near to waterways and watercourses, as well as low-lying drainage swales and creek lines, which could facilitate transfer to waterways during rain events. Additionally, application of the treatment products should not occur immediately before, during or after rain events. If broad-scale treatments are being adopted, allowing several months of degradation time in a catchment area between treatment regimes would reduce the potential for build-up over time and the risks associated with increased chemical mass in the environment.

Environmental monitoring studies of residual concentrations of the anti-parasitical chemicals in dung, soil, plants and water in or near to treatment areas of free-ranging wombats would provide additional information beneficial to understanding how the treatment chemical behave in the Australian environment, and whether they transfer to other environmental matrices (such as plants) or migrate away from treatment areas and wombat toileting areas. The results of these studies could then further inform treatment protocols and restrictions associated with sarcoptic mange in free-ranging wombats.

10.2 Key Recommendations

The following recommendations are made with respect to the topical anti-parasitical chemicals Bravecto® and Cydectin® in free-ranging wombats.

- Adoption of application methods and minimal effective treatment volumes that reduce the chance of spills, run-off and shake-off from wombats.
- Avoiding the use of treatment chemicals near to waterways and watercourses, as well as low-lying drainage swales and creek lines.
- Avoiding, where possible, application immediately prior to or during a rain event. Avoiding application to wet wombats, which may increase the chance of run-off.
- Where broad-scale treatments are being adopted, allow two to three months of degradation time in a catchment area between treatment regimes.
- Undertake research to establish the proportion of active metabolites of moxidectin in wombat dung to improve the understanding of potential risks of Cydectin® to dung and invertebrate soil organisms.
- Develop environmental monitoring studies that involve the collection of dung, soil, plant and water samples in free-ranging wombat treatment areas (including at and near to treated burrow entrances). Time series data on soil would allow for an assessment of degradation (or buildup) of the treatment chemicals over time.

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12 Limitations

The findings of this report are based on the Scope of Work described in this report, consistent with an environmental risk assessment report. Arcadis performed the services in a manner consistent with the standard of care and expertise exercised by members of the environmental profession. That standard of care may change, and new methods and practices of exploration, testing and analysis may develop in the future, which might produce different results.

No warranties, express or implied, are made. Subject to the Scope of Work, Arcadis' assessment is limited strictly to identifying potential environmental risks associated with the nominated chemicals.

While normal assessments of data reliability have been made, Arcadis assumes no responsibility or liability for errors in any data obtained from regulatory agencies, statements from sources outside of Arcadis, or developments resulting from situations outside the scope of this project.

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Appendix A

Environmental Fate Data

Table A-12-1. Summary of Chemical Properties relating to Environmental Fate and Behaviour

	Fluralaner	Ivermectin	Moxidectin
Vapour pressure	Stated as "very low". Quantitative data not found. (EMA, 2016)	<1.5 x 10^{-9} mmHg (Lumaret et al., 2012)	<3.2 x 10^{-8} mmHg (non volatile) (USDA, 2003)
Henry's law constant	No data	4.8 x 10^{-26} (Liebig et al., 2010)	No data
Solubility in water	Insoluble in water (Cayman Chemical, 2023)	2 - 5 mg/L (Liebig et al., 2010; APVMA, 2006)	0.51 mg/L @ 25°C (USDA, 2003) Insoluble in water (PubChem, 2023b) (Zoetis New Zealand Limited, 2019)
log Kow	4.5 – 5.35 (Lewis et al., 2016; Bains et al., 2022)	3.2 – 4.8 (Liebig et al., 2010; Lumaret et al., 2012; Bains et al., 2022)	4.67 - 6 (PubChem, 2023a; EMA, 2017a)
BCF (L/kg)	79.4 L/kg 48.5 L/kg (lipid normalised) (organism not stated) (EMA, 2017b)	100 L/kg (estimated) 52 and 56 L/kg (measured, for avermectin B1a) (Liebig et al., 2010)	Steady state: Low: 2033 L/kg High: 2124 L/kg Steady state corrected for 5% lipid content: Low: 1672 L/kg High: 1745 L/kg Kinetic: Low: 2635 L/kg High: 3093 L/kg Kinetic bioconcentration factor corrected for 5% lipid content: Low: 2162 L/kg High: 2543 L/kg (EMA, 2017a)
Dissociation constant	pKa 12.5 (Bains et al., 2022)	pKa 12.47 (Bains et al., 2022)	pKa 12.8 (weak acid) (PubChem, 2023a)

	Fluralaner	Ivermectin	Moxidectin
UV/VIS absorption (max)	Maxima 265 nm Subject to photolysis (Cayman Chemical, 2023)	Maxima: 237, 245 and 250 nm Subject to direct photolysis (Liebig et al., 2010)	Maxima 243.8 nm Subject to direct photolysis (Awasthi, 2012)
Hydrolysis	Stable at all environmentally relevant pH values at 25°C (Lewis et al., 2016)	Unstable in acidic and basic solutions; most stable at pH 6.3 (Liebig et al., 2010)	No data
Aqueous degradation	Freshwater, aerobic: DT_{50} 3.35 d (river) DT_{50} <7 d (pond) Freshwater, anaerobic: DT_{50} 3.62 d (river) DT_{50} 3.19 d (pond) Aerobic sediments: DT_{50} 85.9 d (river) DT_{50} 71.1 d (pond) Anaerobic sediments DT_{50} 8.53 d (river) DT_{50} 49.1 d (pond) All data reported at 20.8°C (EMA, 2017b)	Dissipation: DT_{50} <0.25 d – 2.9 d (water) DT_{50} 15 - 127 d (whole system) Degradation: $T_{1/2}$ 30 d (water) $T_{1/2}$ 130 d (sediment) $T_{1/2}$ 87 d (whole system) (Liebig et al., 2010) DT_{90} < 16.8 d (whole system) (Lumaret et al., 2012)	Breaks down fairly rapidly through photodegradation. Half-life 6.8 hours (USDA, 2003)
Soil photolysis	No data	DT_{50} <0.5 d (Halley et al., 1993) DT_{50} 3 h (thin dry film exposed to direct sunlight) (Liebig et al., 2010)	Subject to photolysis. (Awasthi, 2012)

	Fluralaner	Ivermectin	Moxidectin
Aerobic soil degradation	Sandy loam: DT_{50} 989 d DT_{90} 3286 d Loam: DT_{50} 404 d DT_{90} 1342 d Clay: DT_{50} 717 d DT_{90} 2382 d Silt loam: DT_{50} 697 d DT_{90} 2315 d (EMA, 2017b)	Madrid soil (61% sand, 15% silt, pH 8.7): DT_{50} 16 d DT_{90} 54 d York soil (69% sand, 13% silt, pH 6.3): DT_{50} 67 d DT_{90} 222 d Artificial soil (75% sand, 17% silt, pH 6.0): DT_{50} 458 d DT_{90} 1520 d (Liebig et al., 2010) Soil half-life: 93 – 240 d (@22°C, dark system, soil/faeces mix) 7 – 14 d (outdoors, summer, soil/faeces mix) 91 – 217 d (outdoors, winter, soil/faeces mix) (Lumaret et al., 2012; Halley et al., 1993)	Sandy loam: DT_{50} 78.6 d @ 20°C Loam: DT_{50} 133.6 d @ 20°C Clay loam: DT_{50} 78.7 d @ 20°C Sand: DT_{50} 139 d @ 20°C DT_{50} 297 d @ 12°C (extrapolated) Mean DT_{50} 104 d @ 20°C and 222 d @ 12°C (EMA, 2017a) DT_{50} 62 d (Lewis et al., 2016) (USDA, 2003).
Soil adsorption / desorption Log Koc (L/kg)	4.3 L/kg (EMA, 2017b)	3.6 – 4.4 L/kg (Liebig et al., 2010; Lumaret et al., 2012) 4.12 L/kg (Heinrich et al., 2021)	4.47 L/kg (Lewis et al., 2016) 4.62 L/kg Buelah loamy sand: 4.45 L/kg Sassafras sandy loam 4.31 L/kg Piano loam 4.27 Silt loam Strong binding to soils (FDA, 2003) 4.74 L/kg (Heinrich et al., 2021)
Animal washoff	Potential for wash-off following topical treatment. Recommendations for dogs are to not swim in watercourses within three days after treatment. (EMA, 2016)		In trials, less than 1% of the applied dose found to wash off treated cattle, when rainfall occurred within 30 mins of application. (USDA, 2003)

	Fluralaner	Ivermectin	Moxidectin
PBT	Very persistent (vP) and toxic (T) but not bioaccumulative (B). Not considered to be a PBT chemical. (EMA, 2017b)	Meets the EU (EC, 2003) P criterion for freshwater sediment but not Australian (refer **Table 6-1** and supporting text). Not bioaccumulative (B), but toxic (T) Not considered to be a PBT chemical. (Liebig et al., 2010)	Very persistent / persistent (vP / P) based on EU, Australian criteria. Toxic (T) and bioaccumulative (B) Considered to be a PBT chemical. (EMA, 2017a)

Appendix B

Environmental Toxicity Data

Table B 12-1. Summary of Environmental Effects Data

	Fluralaner	Ivermectin	Moxidectin
Acute toxicity to mammals	LD_{50} > 2000 mg/kg Single dose *Rattus norvegicus* (EMA, 2017b)	LD_{50} 25 mg/kg *Mus musculus* LD_{50} 50 mg/kg *Rattus norvegicus* LD_{50} 80 mg/kg *Canine familiaris* (Halley et al., 1993)	LD_{50} 84 mg/kg bw (M&F) LD_{50} 42 mg/kg bw (F) *Mus musculus* LD_{50} 106 mg/kg bw (M&F) *Rattus norvegicus* (FDA, 2003; Lewis et al., 2016)
Long- term toxicity to mammals	NOAEL 10 mg/kg Developmental toxicity Foetal body weight *Oryctolagus cuniculus* 28-d NOAEL 60 mg/kg Organ weight *Rattus norvegicus* 52-w NOEL 1 mg/kg Reduction in triglycerides *Canine familiaris* 1-gen NOEL 100 mg/kg Reproduction *Rattus norvegicus* (EMA, 2017b; Wells & Collins, 2022; EMA, 2016)	3 month studies NOEL 0.4 mg/kg/d *Rattus norvegicus* NOEL 0.5 mg/kg/d *Canine familiaris* (Halley et al., 1993)	2-year diet NOEL 5 mg/kg *Mus musculus* 3-generation diet NOEL 0.4 mg/kg *Rattus norvegicus* 52-week diet NOEL 1.1 mg/kg *Canine familiaris* (FDA, 2003)
Acute toxicity to birds	No data	Quantitative data not found. Reported to have low toxicity to birds, chickens and ducks. (APVMA, 2006; Lumaret et al., 2012)	21-d LD_{50} 278 mg/kg *Colinus virginianus* 21-d LD_{50} 365 mg/kg *Anas platyrhynchos* 14-d LD_{50} 283 mg/kg Peterson x Arbor Acres *Gallus gallus domesticus* (Lumaret et al., 2012; FDA, 2003)

	Fluralaner	Ivermectin	Moxidectin
Acute toxicity to fish	LD_{50} > 10 mg/L *Danio rerio* (Wells & Collins, 2022) 96-h LC_{50} >0.049 mg/L *Oncorhynchus mykiss* (Merck, 2023)	96-h LC_{50} 0.003 mg/L *Oncorhynchus mykiss* 96-h LC_{50} 0.017 mg/L *Salmo salar* (juvenile) (Liebig et al., 2010) 96-h LC_{50} 4.8 mg/L *Lepomis macrochirus* 96-h LC50 3.0 mg/L *Salmo gardneri* (Lumaret et al., 2012)	LC_{50} 0.00011 mg/L *Cyprinus carpio* 96-h LC_{50} 0.0002 mg/L *Oncorhynchus mykiss* 96-h LC_{50} 0.0006 mg/L *Lepomis macrochirus* (EMA, 2017a; Lumaret et al., 2012)
Chronic toxicity to fish	21-d NOEC ≥0.049 mg/L *Pimephales promelas* (Merck, 2023)	No data	28-d NOEC $3.2x10^{-6}$ mg/L *Pimephales promelas* (fry survival) (EMA, 2017a; Lumaret et al., 2012)
Acute toxicity to aquatic invertebrates	48-h EC_{50} >0.015 mg/L *Daphnia magna* (Merck, 2023)	48-h EC_{50} $1.2x10^{-6}$ - $10.7x10^{-6}$ mg/L Mean $5.7x10^{-6}$ mg/L *Daphnia magna* 96-h LC_{50} 0.054 mg/L *Palamonetes varians* 96-h LC_{50} 0.000033 mg/L *Gammarus duebeni* and *Gammarus zaddachi* 48-h LC_{50} 0.000026 mg/L *Neomysis integer* (Lumaret et al., 2012) 48-h EC_{50} 0.00059 mg/L *Daphnia magna* (Bundschuh et al., 2015)	48-h EC_{50} 0.00003 mg/L (immobilisation) *Daphnia magna* (EMA, 2017a; Lumaret et al., 2012)

	Fluralaner	Ivermectin	Moxidectin
Chronic toxicity to aquatic invertebrates	NOEC 0.000047 mg/L (reproduction) *Daphnia magna* (Wells & Collins, 2022) 21-d NOEC 0.000074 mg/L *Daphnia magna* (Merck, 2023)	21-d NOEC $0.0003x10^{-6}$ mg/L (growth rate, reproduction and sex ratio) *Daphnia magna* (Lumaret et al., 2012)	21-d NOEC 0.0000031 mg/L 21-d LOEC 0.000025 mg/L (reproduction & parental growth) *Daphnia magna* (EMA, 2017a)
Acute toxicity to sediment dwellers	No data	96-h NOEC 100 µg/kg (reproduction) *Caenorhabditis elegans* (Liebig et al., 2010)	No data
Chronic toxicity to sediment dwellers	No data	10-d NOEC 3.1 µg/kg (larval growth) *Chironomus riparius* 28-d NOEC 160 µg/kg *Lumbriculus variegatus* 224-d NOEC 0.6 µg/kg Nematodes community (Liebig et al., 2010)	28-d NOEC 235 µg/kg (emergence) *Chironomus riparius* (EMA, 2017a)
Toxicity to algae	72-h NOEC ≥0.08 mg/L *Pseudokirchneriella subcapitata** (Merck, 2023)	72-h EC_{50} >4 mg/L (yield, growth rate) *Pseudokirchneriella subcapitata** 72-h LOEC 1.25 mg/L (yield, growth rate) *Pseudokirchneriella subcapitata** 72-h NOEC 0.39 mg/L (yield, growth rate) *Pseudokirchneriella subcapitata** (Liebig et al., 2010)	72-h EC_{50} > 0.11 mg/L 72-h NOEC 0.11 mg/L *Pseudokirchneriella subcapitata** 72-h EC_{50} > 0.0869 mg/L 72-h NOEC 0.0869 mg/L *Raphidocelis subcapitata* (EMA, 2017a)
Oral toxicity to bees	No data	LC_{50} 1.57 µg/mL *Apis mellifera* (Guseman et al., 2016)	LD_{50} 0.46 µg/bee[8] (Zoetis New Zealand Limited, 2019)

[8] Reported in units of µg/bee, but typically oral toxicity to bees is reported in µg/mL. Considered likely to be a typographical error. As the original study was not cited, this has not been confirmed.

	Fluralaner	Ivermectin	Moxidectin
Contact toxicity to bees	No data	LD_{50} 1.32 µg/bee *Apis mellifera* (Mahefarisoa et al., 2021)	LD_{50} 0.025 µg/bee (Zoetis New Zealand Limited, 2019)
Collembolans	No data	28-d EC_{50} 1.7 mg/kg 28-d NOEC 0.3 mg/kg (reproduction) Artificial Soil: TOC 3.6% *Folsomia candida* 28-d EC_{50} 1.7 mg/kg 28-d NOEC 0.3 mg/kg (reproduction) Soil: Total Carbon 1.6% *Folsomia fimetaria* (Liebig et al., 2010) 28-d NOEC 0.4 mg/kg 28-d EC_{50} 0.9 mg/kg (reproduction) Field Soil: TOC 2.2% *Folmosia fimetaria* (Lumaret et al., 2012)	No data
Dung fauna	Topical applications: 48-h LD_{50} 2.86 ng/insect *Haematobia irritans*	21-d EC_{50} 0.0047 mg/kg dung fw *Musca autumnalis* 48-d NOEC 0.00084 mg/kg dung fw (development time) 21-28 d EC_{50} 0.001 mg/kg dung fw (emergence) 28-d LC_{50} 0.021 mg/kg dung fw 48-h LC_{50} 0.036 mg/kg dung fw (larvae) *Scathophaga stercoraria* 21-LC_{50} 0.176 mg/kg fw *Aphodius constans* (Liebig et al., 2010)	EC_{50} 0.47 mg/kg (progeny) NOEC >0.5 mg/kg (adult) NOEC >0.27 mg/kg (progeny) *Euoniticellus intermedius)* LC_{50} 4.0 – 5.4 mg/kg *Aphodius constans* EC_{50} 0.256 mg/kg (progeny) *Digitonthophagus gazella* Moxidectin had no effect on larval survival for *Musca vetustissima*, *Musca domestica*, *Musca inferior* and *Orthelia timorensis*. (Lumaret et al., 2012)

	Fluralaner	Ivermectin	Moxidectin
Other invertebrates	Topical applications: 48-h LD_{50} 65.6 ng/insect *Tribolium castaneum* Oral administration 48-h LD_{50} 1.8 ppm *Drosophila melanogaster* 48-h LD_{50} 12 ppm *Aedes aegypti* (Wells & Collins, 2022) Topical application 24-h LD_{50} 8.62 ng/insect *Solenopsis invicta* (Shao et al., 2023) 48-h LC_{50} 3 ng/insect *Chilo suppressalis* (Sheng et al., 2017) LD_{50} 23.57 mg/kg (oral) *Spodoptera frugiperda* (Zhan et al., 2021)	28-d EC_{50} 36 mg/kg 28-d NOEC 3.0 mg/kg 28-d LC_{50} > 300 mg/kg Field Soil: TOC 1.6% *Enchytraeus crypticus* (Liebig et al., 2010)	EC_{50} 0.134 mg/kg NOEC 0.064 mg/kg *Haematobia irritans exigua* (Lumaret et al., 2012)

	Fluralaner	Ivermectin	Moxidectin
Toxicity to earthworms	No data	56-d EC_{50} 5.3 mg/kg (reproduction) 28-d NOEC 2.5 mg/kg (reproduction) Artificial Soil: TOC 3.6% *Eisenia foetida* 28-d NOEC 12 mg/kg (biomass) 28-d LC_{50} 315 mg/kg Artificial Soil *Eisenia foetida* 14-d NOEC 4 mg/kg (biomass) 14-d LC_{50} 15.8 mg/kg Artificial Soil *Eisenia foetida* (Liebig et al., 2010) 14-d EC_{50} 4.0 mg/kg (Cocoon production) 14-d NOEC 4.0 mg/kg (biomass) Artificial soil *Eisendia foetida* (Lumaret et al., 2012)	28-d LC_{50} 37.2 mg/kg 28-d NOEC 20 mg/kg (mortality) 28-d NOEC 0.84 mg/kg (reproduction) *Eisenia foetida* (EMA, 2017a)
Effects on seedling emergence	No data	No data	Reduced seedling emergence at 0.1% moxidectin. (Eichberg et al., 2016) No observed phytotoxicity: at 4 kg/ha (FDA, 2003)
Effects on vegetative vigour	No data	NOEC 0.56 mg/kg (Lumaret et al., 2012)	$NOEC_{phytotoxicity}$ 4 kg/ha *Abutilon theophrasti, Ambrosia artemisiifolia, Avena fatua, Brassica kaber, Calystegia arvensis, Cyperus rotundus, Digitaria sanguinalis, Echinochloa crusgalli, Elytrigia repens (quackgrass), Ipomoea sp., Setaria viridis, Sida spinosa.* (Lumaret et al., 2012)

	Fluralaner	Ivermectin	Moxidectin
Effects on soil micro-organisms	No data	Soil containing 30 µg/kg of ivermectin and metabolites in dung from ivermectin-treated steers showed no effect upon nitrification or respiration. (Halley et al., 1993)	No data

* *Pseudokirchneriella subcapitata* now known as *Raphidocelis subcapitata*

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