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Comparison of the environmental properties of parasiticides and harmonisation of the basis for environmental assessment at the EU level

by

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Abstract

Avermectin and milbemycin parasiticides have a high toxicity to non-target organisms, are often persistent and may have a potential to bioaccumulate. The present project contributes to filling gaps in the database for a complete environmental risk assessment of these parasiticides. In addition, risk management strategies for parasiticides used in pasture animals were discussed. For ivermectin and selamectin, log POW values of 5.6 and 6.0 were derived, respectively. In studies with zebrafish, bioconcentration factors of 63–111 for ivermectin and 70–71 for doramectin (based on total radio-active residues, normalised to a 5% lipid content) were determined. Generally, about 90% of the avermectins and milbemycins applied to pasture animals are excreted within approx. 4–10 days after application, but the parasiticides can be detected for much longer in the faeces. Doramectin is most toxic to dung organisms, followed by ivermectin and eprinomectin having a similar toxicity, and moxidectin. The evaluated risk management strategies include sustainable approaches to control parasites, risk mitigation measures (RMMs) and restrictions of use. Parasiticides are a central component of strategies to control parasites. Yet, their prudent use is generally recommended. Treatment frequencies should be reduced to the minimum required to sufficiently control parasitoses. Where indicated, selective or targeted selective treatments should be used instead of strategic treatments. Six RMMs were evaluated with regard to their efficacy to reduce the risk for dung or soil organisms, and their practicability. For most of these RMMs, data gaps were identified that have to be filled in order to sufficiently specify the measures and to fully evaluate their suitability and practicability. Since most of the RMMs have the potential to contribute to a reduction of the environment risk caused by avermectins and milbemycins, a further development / specification is recommended.

Kurzbeschreibung

Parasitizide aus den Gruppen der Avermectine und Milbemycine haben eine hohe Toxizität gegenüber Nichtzielorganismen, sind oft persistent und potenziell bioakkumulierend. Das vorliegende Projekt trägt dazu bei, Lücken in der Datenbasis für eine vollständige Umweltrisikobewertung dieser Parasitizide zu füllen. Außerdem wurden Risikomanagementstrategien für in Weidetieren eingesetzte Parasitizide diskutiert. Für Ivermectin und Selamectin wurden log P_{OW}-Werte von 5,6 bzw. 6,0 bestimmt. In Tests mit Zebrabärblingen wurden Biokonzentrationsfaktoren von 63–111 für Ivermectin und 70–71 für Doramectin ermittelt (basierend auf der Gesamtradioaktivität, normalisiert auf einen Lipidgehalt von 5%). In Weidetieren werden im Allgemeinen etwa 90% der verabreichten Avermectine und Milbemycine innerhalb von ca. 4–10 Tagen nach Applikation ausgeschieden. Die Parasitizide sind jedoch deutlich länger in den Fäzes nachweisbar. Doramectin hat die höchste Toxizität gegenüber Dungorganismen, gefolgt von Ivermectin und Eprinomectin, deren Toxizität vergleichbar ist, und Moxidectin. Die evaluierten Risikomanagementstrategien umfassen nachhaltige Herangehensweisen zur Parasitenkontrolle, Risikominderungsmaßnahmen (RMM) und Anwendungsbeschränkungen. Parasitizide sind ein zentraler Bestandteil von Strategien zur Parasitenkontrolle. Sie sollten jedoch stets umsichtig eingesetzt werden. Behandlungsfrequenzen sollten auf das zur Kontrolle von Parasitosen notwendige Minimum reduziert werden. Soweit möglich sollten strategische Behandlungen durch selektive oder gezielte, selektive Behandlungen ersetzt werden. Sechs RMM wurden in Hinblick auf ihre Effektivität, das Risiko für Dung- bzw. Bodenorganismen zu reduzieren, und ihre Praktikabilität bewertet. Für die meisten dieser Maßnahmen wurden Datenlücken identifiziert, die gefüllt werden müssen, um die Maßnahmen ausreichend zu spezifizieren und anschließend ihre Effektivität und Praktikabilität vollständig bewerten zu können. Da die meisten RMM dazu beitragen können, durch Avermectine und Milbemycine verursachte Umweltrisiken zu reduzieren, wird eine Weiterentwicklung / Spezifizierung der RMM empfohlen.

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List of Abbreviations

AF	Accumulation factor
B	Bioaccumulation
BCF	Bioconcentration factor
BCF_k	Kinetic bioconcentration factor
BCF_{Kg}	Growth-corrected kinetic bioconcentration factor
BCF_{KgL}	Lipid-normalised growth-corrected kinetic bioconcentration factor
BCF_{KgLpc}	Lipid-normalised and growth-corrected kinetic BCF of parent compound
BCF_{KL}	Lipid-normalised kinetic bioconcentration factor
BCF_{KLpc}	Lipid-normalised kinetic bioconcentration factor (BCF_K) of parent compound
BCF_{Kpc}	Kinetic bioconcentration factor (BCF_K) of parent compound
BCF_{SS}	Bioconcentration factor at steady state
BCF_{SSL}	Lipid normalised bioconcentration factor at steady state
BCF_{SSLpc}	Lipid-normalised steady-state bioconcentration factor (BCF_{ss}) of parent compound
BCF_{SSpc}	Steady-state bioconcentration factor (BCF_{ss}) of parent compound
BfN	German Federal Agency for Nature Conservation
BMF	Biomagnification factor
bq	Becquerel
bw	Body weight
C_f	Concentration of the test substance in fish
C_O	Concentration of the test substance in 1-octanol
C_W	Concentration of the test substance in water
d	Day
dpm	Disintegrations per minute
dw	Dry weight
EM(E)A	European Medicines Agency
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
Fh-IME	Fraunhofer-Institute for Molecular Biology and Applied Ecology
fw	Fresh weight
GDR	German Democratic Republic
HMA	European Heads of Medicines Agencies
HPLC	High performance liquid chromatography
i.m.	Intramuscular injection
k_1	Uptake rate constant

k_2	Depuration rate constant
k_{2g}	Growth-corrected depuration rate constant
k_g	Growth rate constant
LOD	Limit of detection
LOQ	Limit of quantification
LSC	Liquid scintillation counting
log P_{OW}	Logarithm of the octanol/water partition coefficient
log $P_{OW, Av}$	Average log P_{OW} value
MRL	Maximum residue limits
NGO	Non-governmental organisation
NOEC	No observed effect concentration
OECD	Organisation for Economic Cooperation and Development
P	Persistence
PEC	Predicted environmental concentration
PNEC	Predicted no effect concentration
P_{OW}	Octanol/water partition coefficient
PRP	Percentage of radioactivity associated with the parent compound
R	Coefficient of correlation
R^2	Coefficient of determination
RP-HPLC	reversed-phase high performance liquid chromatography
RMM	Risk mitigation measure
s.c.	Subcutaneous injection
SPC	Summary of product characteristics
T	Toxicity
TG	Test guideline
TLC	Thin layer chromatography
TRR	Total radioactive residues
UBA	German Environment Agency (Umweltbundesamt)

Summary

Introduction

Parasiticides belonging to the avermectins and milbemycins have a high toxicity to non-target organisms in the aquatic and terrestrial environment. In addition, many of these parasiticides are known to be persistent and may have a potential to bioaccumulate. Although these substances are on the market since decades, a complete environmental risk assessment including an assessment of persistence (P), bioaccumulation (B) and toxicity (T), is often not possible, because some data are lacking. Moreover, a number of products containing avermectins or milbemycins were authorised, although a high environmental risk was identified. In the summaries of product characteristics of these products, risk mitigation measures are described that aim at reducing the environmental risk. However, it has often been criticised that these measures are not feasible with regard to the agricultural practice.

The present project is filling gaps in the database for a complete environmental risk assessment by (1) deriving octanol/water partition coefficients for ivermectin and selamectin, and fish bioconcentration factors (BCF) for ivermectin and doramectin, and (2) evaluating publicly available data on the excretion of avermectins and milbemycins by pasture animals, and on the effects of these parasiticides on dung organisms. In addition, it contributes to a further evaluation of risk management strategies for parasiticides (avermectins and milbemycins) used in pasture animals (cattle, horses, sheep) by compiling and evaluating (a) sustainable approaches to control parasites in pasture animals and (b) risk mitigation measures aiming at the protection of dung and soil organisms. The project results were discussed during a workshop with representatives from competent authorities and industry, veterinarians and farmers.

Octanol/water partition coefficients and bioconcentration factors

Octanol/water partition coefficients were determined using the slow stirring method according to OECD test guideline 123, which is appropriate for substances with expected log P_{OW} values above 4. In three replicate experiments, average log P_{OW} values of 6.0 ± 0.7 for selamectin and 5.6 ± 0.3 for ivermectin were derived.

Bioconcentration tests with zebrafish (*Danio rerio*) were performed according to OECD test guideline 305 using radiolabelled (^{3}H) ivermectin and doramectin. Based on the results of a pre-test, the definitive test with ivermectin was carried out at two concentrations (0.01 and 0.10 µg/L). It included a 20-day uptake phase and a subsequent depuration phase of 10 days. For doramectin, a single concentration (0.041 µg/L) was used in the definitive test, which consisted of a 24-day uptake and an 11-day depuration phase. The determined bioconcentration factors ranged from 63–111 for ivermectin and 70–71 for doramectin (related to total radioactive residues and normalised to a 5% lipid content). These BCF values are clearly below the threshold value of 2000 for the B-criterion specified in Annex XIII of the REACH regulation.

Excretion of avermectins and milbemycins by pasture animals

Publicly available excretion data were collected for three avermectins (ivermectin, doramectin, eprinomectin) and one milbemycin (moxidectin) that are authorised in Germany for the treatment of pasture animals (cattle, sheep, horses). The data were evaluated with regard to the amount of excreted substance relative to the applied dose, the time-point of maximum faecal excretion, the total duration of the faecal excretion and possible metabolites. Avermectins are only marginally metabolised in pasture animals, while moxidectin is metabolised to a larger extent. Both avermectins and milbemycins are primarily excreted with the faeces. The excretion rates depend on a number of factors including the animal species, breed and age, as well as the route of administration, formulation and dosage of the

parasiticide. In general, about 90% of the applied dose are excreted within approx. 4 to 10 days after application. Yet, the parasiticides can be detected for much longer periods (in some cases more than 100 days) in the faeces of the treated animals.

Effects of avermectins and milbemycins on dung organisms

Information on the toxicity of avermectins and milbemycins to dung flies and dung beetles was compiled based on available reviews, a search of recent literature and own recent studies. In addition to the active substances authorised for use in pasture animals (ivermectin, doramectin, eprinomectin, moxidectin), possible alternatives (avermectin B_1, emamectin, selamectin, milbemycinoxim) were considered. Overall, doramectin has the highest toxicity to dung organisms, followed by ivermectin and eprinomectin that show a similar toxicity. Moxidectin is the least toxic of these four parasiticides. For doramectin, eprinomectin, avermectin B_1, and especially emamectin, selamectin and milbemycinoxim, information on the toxicity to dung organisms is very scarce or even lacking.

Risk management strategies for parasiticides used in pasture animals

Possible risk management strategies for parasiticides used to treat pasture animals were compiled and discussed. Focus was mainly placed on ivermectin, doramectin, eprinomectin and moxidectin, parasiticides fulfilling some or (in case of moxidectin) all PBT criteria. The risk management strategies include sustainable approaches to control parasites, risk mitigation measures and possible restrictions of use.

Sustainable approaches to control parasites

Optimised treatment regimes, a good management of grazing land and good animal husbandry practices are important aspects of sustainable approaches to control parasites. Due to animal welfare considerations and the epidemiology of relevant parasite species in the different pasture animals, parasiticides are an essential component of strategies to control parasites. However, a prudent use of antiparasitics is generally recommended. Within integrated treatment programmes, which include complementary prophylactic measures such as an appropriate management of grazing land, the frequencies of antiparasitic treatments should be reduced to the minimum required to sufficiently control parasitoses. In addition to reducing the effects on non-target organisms, such an approach would help to prevent the further development of parasiticide resistances. Strategically useful times of treatment should be selected to cause a lasting disruption of the developmental cycle of the parasite. The success of antiparasitic treatments should be evaluated regularly using e.g. an egg count reduction test. In view of the diversity of the situation, which involves the treatment of different pasture animal species, breeds and age classes, different parasites, various epidemiological situations, animal husbandry and farming systems, case-specific approaches are needed for an effective control of parasites. Within the present project, general aspects were addressed as outlined in the following.

Sufficiently large refugia, in which susceptible parasites survive, should be preserved to prevent the further development and distribution of parasiticide resistances. Moreover, a low infection pressure on the pasture is desirable, since it leads to the development of a protective immunity within the livestock animals.

Where possible and indicated, selective treatments or targeted selective treatments should be used instead of strategic treatments, i.e. only a part of the herd should be treated, while the other animals should remain untreated. If these treatment approaches are applied, lower amounts of parasiticides are used, and refugia for susceptible parasites and dung organisms are available. The success of selective treatment approaches depends on the training of the farmers, the communication between veterinarians and farmers, and on an appropriate clinical, epidemiological, and diagnostic evaluation of each specific situation. Generally, selective treatment approaches are feasible, if the animals that have to be treated can be identified and the optimal times for diagnosis and therapy can be selected. There is still

a need for research on indicators that can be used to decide, whether a treatment is required and when this treatment should be performed. Non-immune young animals, which are for the first time on the pasture, are particularly threatened by parasites and have to be treated strategically at specific intervals. To reduce the infection pressure, young animals should preferably spend their first grazing season with older animals on pastures with a low stocking density. Where possible, they should be moved regularly to a new pasture or plot.

Pasture animals (especially first year grazing animals) have to be treated when the infection pressure is high. For this reason, there are only limited possibilities to reduce the amount of parasiticides excreted to the pasture by shifting the time and, consequently, place of treatment with a parasiticide (i.e. by treating animals before being turned out to pasture and after their return to the stable). A detailed analysis is required for each pasture animal species, parasite and antiparasitic product.

At present, no anthelmintic vaccine is available in Germany. Further research efforts are required with regard to vaccination and other possible alternative measures (e.g. breeding programmes, nematophagous fungi and condensed tannins) that might contribute to control parasites in pasture animals.

A compilation and evaluation of data on the prevalence of parasites on farms, the actual use of parasiticides, the success of antiparasitic treatments and the resistance situation could contribute to further develop recommendations for selection of the most suitable strategy to control parasitoses, combining optimised antiparasitic treatments and complementary measures.

Risk mitigation measures

Risk mitigation measures (RMMs) for pasture animals were compiled based on EM(E)A documents, the results of previous projects and a supplementary literature search. Six measures (three RMMs for the protection of dung organisms, and three RMMs for the protection of soil organisms) were selected for evaluation and discussion of their efficacy to reduce the risk for dung or soil organisms, and their practicability.

The first three measures listed in the following aim at protecting dung organisms. The last three measures aim at the protection of soil organisms.

RMM: Strategic treatment of the animal group/herd is only allowed outside the periods of maximal abundance and diversity of dung organisms

The present knowledge on the biology and ecology of dung flies and dung beetles is insufficient to identify appropriate time windows, during which parasiticides could be administered to pasture animals without harming dung organisms. Currently, it appears unlikely that time windows will be identified, which are appropriate for treating pasture animals and during which dung organisms are inactive. However, it is recommended to critically check and, where possible, reduce current frequencies of antiparasitic treatments. If possible and indicated, selective treatments or targeted selective treatments should be used instead of strategic treatments.

To evaluate the practicability of the RMM, comprehensive data are required on the time / frequency of application of each parasiticide in the different pasture animal species, breeds and age classes for each farming method / husbandry system. Restrictions of the time, during which a parasiticide can be applied, have to be made for each livestock species and indication in close cooperation with parasitologists.

RMM: The product is toxic to dung organism (flies, beetles). Therefore, do not treat animals on the same pasture in successive seasons to avoid adverse effects on dung fauna and their predators

This measure is suitable to protect multivoltine dung organisms. Whether univoltine species would benefit from the RMM, depends on the overlap of their reproductive cycle and the time of antiparasitic treatment. The measure appears generally practicable for cattle, horses and sheep, with its practicability mainly depending on the availability of sufficiently large pasture areas allowing the implementa-

tion of a rotational grazing scheme. If and to which extent the RMM can be implemented in routine farming practices, has to be evaluated for each farm animal species and farming system. When specifying this RMM, other parasiticides with the same or a similar mode of action should also be considered. This means that it should be avoided to treat animals on the same pasture during successive seasons with different active ingredients having the same or a similar mode of action.

RMM: Animals from free-range husbandry must be stabled during treatment and for X days following treatment

Dung organisms would benefit from this RMM, if the farm animals can be stabled for a sufficiently long period. However, to protect the most sensitive dung organisms (especially Sepsidae), this period may be impracticably long. Yet, stabling the animals during the period of peak excretion of the parasiticide would reduce exposure of dung organisms in the environment considerably. The measure is feasible for farming systems, where the animals are not kept on pastures all-year-round, if the period during which the animals have to be stabled is not too long and the pastures are relatively close to the stables. The RMM has to be specified for each parasiticide product, administration route, dose, farm animal species and breed. More information is needed on the ecology of the most important dung organism groups (e.g. duration of life cycles, horizontal distribution) and on their sensitivity towards parasiticides.

RMM: Manure from treated animals must be stored for X months prior to spreading on and incorporating into land to allow for degradation of the active substance prior to release into the environment

Soil organisms would benefit from this RMM, if the manure can be stored long enough, so that the antiparasitic is degraded to a sufficient extent. The measure can be applied to liquid manure or dung that is stored before spreading to land, i.e. to manure that is in most cases generated by animals that are stabled (either temporarily or throughout the year) and treated during this stabling period. Hence, it is relevant for cattle and horses, but generally not for sheep. Whether the measure is practicable, depends on the required storage time for manure of the respective farm animal species containing the parasiticide. The RMM has to be specified for each parasiticide based on its half-life time (DT_{50}) in stored liquid or solid manure of the respective farm animal species. However, at present such DT_{50} values are not publicly available.

RMM: When spreading liquid or solid manure from treated animals onto arable land, the maximum nitrogen spreading limit must not exceed X kg N per hectare and year (X < 170)

This measure is suitable to protect soil organisms. It can be applied liquid manure or dung that spread to land and is thus relevant for cattle and horses, but in most cases not for sheep. It is practicable, if sufficiently large agricultural areas are available that can be used for application of the manure. In regions, where farm animals are intensively kept, it might be difficult to find enough sites where the manure could be spread. If manure is sold, it has to be ascertained that information on the reduced maximal amount of nitrogen to be applied per hectare and year is passed on from the farmer selling the manure to the farmer applying the manure. The RMM needs to be specified for each parasiticide product, animal species, dosage, application frequency and manure-spreading scenario. When specifying this RMM, other parasiticides with the same or a similar mode of action should also be considered.

RMM: Manure containing the active substance should not be spread on the same area of land in successive years to avoid accumulation of the active substance, which may cause adverse effects on the environment

This RMM is appropriate to reduce the accumulation of a parasiticide in soil and, thus, the exposure of soil organisms. A consistent approach based on a $DT_{50\ soil} > 120$ days, a $DT_{90\ soil} > 1$ year and / or a $PEC_{soil\ plateau}$ to PNEC ratio ≥ 1 should be used to decide whether this RMM should be implemented for a parasiticide. The RMM can be applied to manure spread to land and is, thus, relevant for cattle and horses but typically not for sheep. The RMM is practicable, if sufficiently large agricultural areas are

available that can be used for application of the manure. In regions, where farm animals are intensively kept, it might be difficult to find enough sites where the manure could be spread. If manure is sold, it has to be ascertained that information on the parasiticide used to treat the animals that have produced the manure, is passed on from the farmer selling the manure to the farmer applying the manure. When specifying this RMM, other parasiticides with the same or a similar mode of action should also be considered. This means that it should be avoided to spread manure containing different active ingredients having the same or a similar mode of action on the same area of land in successive years.

Possibilities to restrict the use of authorised parasiticides

The number of parasiticides authorised in Germany for the treatment of pasture animals is relatively small, and the perspectives for the development of new active ingredients are limited. In view of these facts and the current resistance situation, the replacement of an environmentally problematic active substance by an active substance with similar efficiency towards target organisms but a reduced risk to the environment appears difficult.

Summary of the evaluation of risk management strategies

Currently, parasiticides appear indispensable to effectively control parasitoses in pasture animals. Their prudent use is considered as most promising approach to reduce negative effects on dung and soil organisms. A crucial point is to minimise the use of parasiticides by replacing strategic treatments by selective or targeted selective treatments where feasible.

Risk mitigation measures may contribute to reducing the risk for dung and soil organism communities. However, for most of the evaluated RMMs data gaps were identified that have to be filled to sufficiently specify the respective measure and to fully evaluate its suitability and practicability. Such an evaluation has to be performed for each parasiticide product and livestock species. In this context, it should be pointed out that even if a measure can only be applied under certain conditions (depending for instance on the animal husbandry and farming system), it may still contribute considerably to reducing the environmental risk.

A number of knowledge gaps and associated research needs were identified that are related to dung organism biology / ecology, and the effects of parasiticides on dung organism communities, and the further development of sustainable approaches to control parasites and risk mitigation measures.

Finally, it should be pointed out that the current economic situation of farmers is a major factor limiting the practicability of a number of approaches outlined in the present report.

Zusammenfassung

Einführung

Parasitizide aus den Gruppen der Avermectine und Milbemycine haben eine hohe Toxizität gegenüber Nichtzielorganismen in der aquatischen und terrestrischen Umwelt. Sie sind oft persistent und können potenziell bioakkumulierend sein. Obwohl diese Parasitizide schon seit Jahrzehnten auf dem Markt sind, ist eine vollständige Umweltrisikobewertung – einschließlich einer Bewertung der Persistenz (P), Bioakkumulation (B) und Toxizität (T) – oft nicht möglich, da einige Daten fehlen. Etliche Avermectine oder Milbemycine enthaltende Produkte wurden außerdem trotz eines identifizierten hohen Umweltrisikos zugelassen. In den Zusammenfassungen der Merkmale dieser Parasitizide werden Risikominderungsmaßnahmen beschrieben, mit denen das Umweltrisiko reduziert werden soll. Es wurde jedoch oft kritisiert, dass diese Maßnahmen in der landwirtschaftlichen Praxis nicht durchführbar sind.

Das vorliegende Projekt trägt dazu bei, vorhandene Lücken in der Datenbasis für eine vollständige Umweltrisikobewertung zu füllen: Oktanol/Wasser-Verteilungsquotienten (P_{OW}) für Ivermectin und Selamectin sowie Fisch-Biokonzentrationsfaktoren (BCF) für Ivermectin und Doramectin wurden ermittelt. Daten zur Exkretion von kommerziell verfügbaren Avermectinen und Milbemycinen durch Weidetiere und zu den Effekten dieser Wirkstoffe auf Dungorganismen wurden ausgewertet. Das Projekt trägt außerdem zu einer Weiterentwicklung von Risikomanagementstrategien für Parasitizide (Avermectine und Milbemycine) bei, die Weidetieren (Rinder, Pferde, Schafe) verabreicht werden: Nachhaltige Herangehensweisen zur Kontrolle von Parasiten bei Weidetieren und Risikominderungsmaßnahmen zum Schutz von Dung- und Bodenorganismen wurden zusammengetragen und bewertet. Die Projetergebnisse wurden auf einem Workshop mit Vertretern von Behörden und Industrie sowie Tierärzten und Landwirten diskutiert.

Oktanol/Wasser-Verteilungsquotienten und Biokonzentrationsfaktoren

Oktanol/Wasser-Verteilungsquotienten wurden mit der Methode zur Prüfung unter langsamem Rühren nach OECD-Testrichtlinie 123 bestimmt, die sich für Substanzen mit erwarteten log P_{OW}-Werten über 4 eignet. In drei parallelen Versuchen wurden durchschnittliche log P_{OW}-Werte von 6,0 ± 0,7 für Selamectin und 5,6 ± 0,3 für Ivermectin ermittelt.

Biokonzentrationstests mit Zebrabärblingen (*Danio rerio*) wurden nach OECD-Testrichtlinie 305 mit radioaktiv markiertem (^{3}H) Ivermectin und Doramectin durchgeführt. Aufgrund der Ergebnisse des Vortests wurden im Haupttest mit Ivermectin zwei Substanzkonzentrationen (0,01 und 0,10 µg/L) eingesetzt. Der Haupttest bestand aus einer 20-tägigen Aufnahme- und einer 10-tägigen Eliminationsphase. Für Doramectin wurde der Haupttest mit einer Substanzkonzentration (0,041 µg/L) durchgeführt; er bestand aus einer 24-tägigen Aufnahme- und einer 11-tägigen Eliminationsphase. Es wurden Biokonzentrationsfaktoren von 63–111 für Ivermectin und 70–71 für Doramectin ermittelt (basierend auf der Gesamtradioaktivität, normalisiert auf einen Lipidgehalt von 5%). Diese BCF-Werte liegen deutlich unter dem Schwellenwert von 2000 für das B-Kriterium, der in Annex XIII der REACH-Verordnung festgelegt ist.

Exkretion von Avermectinen und Milbemycinen durch Weidetiere

Für drei Avermectine (Ivermectin, Doramectin, Eprinomectin) und ein Milbemycin (Moxidectin), die in Deutschland für die Behandlung von Weidetieren (Rinder, Schafe und Pferde) zugelassen sind, wurden öffentlich verfügbare Exkretionsdaten recherchiert. Diese Daten wurden in Hinblick auf die Exkretion des nicht metabolisierten Wirkstoffs (%, bezogen auf die verabreichte Wirkstoffmenge), den Zeitpunkt der maximalen fäkalen Exkretion, die Gesamtdauer der fäkalen Exkretion sowie mögliche Metaboliten

ausgewertet. Avermectine werden in Weidetieren kaum metabolisiert, während Moxidectin stärker metabolisiert wird. Sowohl Avermectine als auch Moxidectin werden primär über die Fäzes ausgeschieden. Die Exkretionsraten hängen von etlichen Faktoren ab (u.a. Tierart und -rasse, Alter der Tiere, Verabreichungsweg, Wirkstoffformulierung und –dosierung). Im Allgemeinen werden etwa 90% der verabreichten Dosis innerhalb von 4 bis 10 Tagen nach der Applikation ausgeschieden. Die Parasitizide können jedoch über einen deutlich längeren Zeitraum (z.T. über mehr als 100 Tage) in den Fäzes der behandelten Tiere nachgewiesen werden.

Auswirkungen von Avermectinen und Milbemycinen auf Dungorganismen

Daten zu den Effekten von Avermectinen und Milbemycinen auf Dungfliegen und -käfer wurden basierend auf Übersichtsarbeiten, einer Recherche neuerer Literatur und vorliegenden eigenen Studien zusammengestellt. Zusätzlich zu den in Weidetieren zugelassenen Wirkstoffen (Ivermectin, Doramectin, Eprinomectin, Moxidectin) wurden auch mögliche Alternativen (Avermectin B_1, Emamectin, Selamectin, Milbemycinoxim) berücksichtigt. Insgesamt hat Doramectin die höchste Toxizität für Dungorganismen, gefolgt von Ivermectin und Eprinomectin, deren Toxizität vergleichbar ist, und Moxidectin, das von diesen vier Wirkstoffen die niedrigste Toxizität hat. Für Doramectin, Eprinomectin, Avermectin B_1, und besonders Emamectin, Selamectin und Milbemycinoxim liegen nur sehr wenige bzw. gar keine Daten zu den Effekten auf Dungorganismen vor.

Risikomanagementstrategien für in Weidetieren eingesetzte Parasitizide

Mögliche Risikomanagementstrategien für Antiparasitika, die zur Behandlung von Weidetieren verwendet werden, wurden zusammengetragen und diskutiert. Dabei lag der Schwerpunkt auf Ivermectin, Doramectin, Eprinomectin und Moxidectin, d.h. Parasitiziden, die einige oder (im Fall von Moxidectin) alle PBT-Kriterien erfüllen. Die diskutierten Risikomanagementstrategien umfassen nachhaltige Herangehensweisen zur Kontrolle von Parasiten, Risikominderungsmaßnahmen und die Möglichkeit von Anwendungsbeschränkungen.

Nachhaltige Herangehensweisen zur Kontrolle von Parasiten

Optimierte Behandlungsschemata, ein gutes Weidemanagement und gute Tierhaltungspraktiken sind wichtige Aspekte nachhaltiger Herangehensweisen zur Kontrolle von Parasiten. Aus Tierschutzgründen und in Anbetracht der Epidemiologie relevanter Parasitenarten in den verschiedenen Weidetieren sind Parasitizide ein essentieller Bestandteil von Strategien zur Parasitenkontrolle. Sie sollten jedoch stets umsichtig eingesetzt werden. Im Rahmen von integrierten Behandlungsprogrammen, die ergänzende prophylaktische Maßnahmen wie z.B. ein geeignetes Weidemanagement beinhalten, sollten Behandlungsfrequenzen auf das zur Kontrolle von Parasitosen notwendige Minimum reduziert werden. Eine solche Herangehensweise würde zum einen die Effekte auf Nichtzielorganismen reduzieren und zu anderen dazu beitragen, der weiteren Ausbreitung von Resistenzen gegenüber Antiparasitika vorzubeugen. Parasitizide sollten an strategisch sinnvollen Zeitpunkten eingesetzt werden, um die Entwicklungszyklen von Parasiten möglichst nachhaltig zu stören. Außerdem ist eine regelmäßige Überprüfung der Wirksamkeit von antiparasitären Behandlungen (z.B. mit einem Eizahl-Reduktions-Test) wünschenswert. Angesichts der insgesamt sehr heterogenen Situation (verschiedene Weidetierarten,
-rassen, Altersklassen, Parasiten, epidemiologische Situationen, Tierhaltungspraktiken, landwirtschaftliche Methoden u.a.) ist die Erarbeitung von entsprechend differenzierten Vorschlägen für nachhaltige Herangehensweisen zur Parasitenkontrolle notwendig. Im Rahmen des vorliegenden Projekts wurden die folgenden allgemeinen Aspekte betrachtet.

Um die weitere Entwicklung und Ausbreitung von Antiparasitika-Resistenzen zu verhindern, sollten ausreichend große Refugien, in denen empfindliche (d.h. nicht resistente) Parasiten überleben, erhal-

ten werden. Ein geringer Infektionsdruck ist zudem wünschenswert, da er die Entwicklung einer schützenden Immunität der Weidetiere fördert.

Strategische Behandlungen sollten soweit möglich durch selektive oder gezielte, selektive Behandlungen ersetzt werden, d.h. nur ein Teil der Herde sollte behandelt werden, während die restlichen Tiere unbehandelt bleiben. Auf diese Weise kann die Menge der eingesetzten Parasitizide verringert werden und es stehen Refugien für empfindliche Parasiten und Dungorganismen zur Verfügung. Der Erfolg dieser selektiven Behandlungsmethoden hängt von der Ausbildung der Landwirte, der Kommunikation zwischen Tierärzten und Landwirten und der adäquaten klinischen, epidemiologischen und diagnostischen Bewertung jeder spezifischen Situation ab. Selektive Behandlungsmethoden sind im Allgemeinen dann praktikabel, wenn die zu behandelnden Tiere und der optimale Zeitpunkt für eine Behandlung identifiziert werden können. Hinsichtlich geeigneter Behandlungsindikatoren besteht allerdings noch Forschungsbedarf. Junge, noch nicht immune Weidetiere reagieren während ihrer ersten Weideperiode sehr empfindlich auf Parasitenbefall und müssen in bestimmten Zeitabständen strategisch behandelt werden. Um den Infektionsdruck zu reduzieren, sollte Jungtiere ihre erste Weidesaison zusammen mit älteren Tieren auf Weiden mit geringer Besatzdichte verbringen, wenn möglich mit regelmäßigem Umtrieb.

Weidetiere, besonders die oben erwähnten jungen Tiere während ihrer ersten Weideperiode, müssen behandelt werden, wenn der Infektionsdruck hoch ist. Daher gibt es nur begrenzte Möglichkeiten, die Menge der auf die Weide exkretierten Parasitizide durch eine Verschiebung des Zeitpunkts und damit auch des Orts der antiparasitären Behandlung zu reduzieren, indem Tiere vor dem Weideaustrieb und nach der Rückkehr in den Stall behandelt werden. Hier ist eine detaillierte Analyse für jede Nutztierart, jeden Parasiten und jedes Antiparasitikum erforderlich.

Zurzeit steht in Deutschland kein Impfstoff gegen Helminthen zur Verfügung. In diesem Bereich und in Bezug auf weitere alternative Herangehensweisen (z.B. Zuchtprogramme, nematophage Pilze, kondensierte Tannine), die dazu beitragen könnten, Parasiten zu kontrollieren, besteht Forschungsbedarf.

Eine Erfassung und Auswertung von Daten zur Prävalenz von Parasiten auf Viehhaltungsbetrieben, zur tatsächlichen Verwendung von Parasitiziden, zum Erfolg antiparasitärer Behandlungen und zur Resistenzsituation könnte dazu beitragen, Empfehlungen zur Auswahl der geeignetsten Strategie zur Parasitenkontrolle weiterzuentwickeln. Eine solche Strategie sollte neben antiparasitären Behandlungen auch komplementäre Maßnahmen enthalten.

Risikominderungsmaßnahmen

Risikominderungsmaßnahmen (RMM) für Weidetiere wurden basierend auf EM(E)A-Dokumenten, den Ergebnissen vorheriger Projekte und einer ergänzenden Literaturrecherche zusammengestellt. Sechs Maßnahmen (drei RMM zum Schutz von Dungorganismen und drei RMM zum Schutz von Bodenorganismen) wurden ausgewählt und in Hinblick auf ihre Effektivität, das Risiko für Dung- bzw. Bodenorganismen zu reduzieren, und ihre Praktikabilität bewertet und diskutiert.

Die ersten drei der im Folgenden genannten Maßnahmen zielen auf den Schutz von Dungorganismen ab. Durch die letzten drei Maßnahmen sollen Bodenorganismen geschützt werden.

RMM: Strategische Behandlungen von Tiergruppen/Herden nur außerhalb der Populations- bzw. Diversitätsmaxima von Dungorganismen durchführen

Das vorliegende Wissen zur Biologie und Ökologie von Dungfliegen und -käfern reicht nicht aus, um geeignete Zeitfenster zu identifizieren, in denen Weidetieren Parasitizide verabreicht werden können, ohne Dungorganismen zu schädigen. Zurzeit erscheint es relativ unwahrscheinlich, dass Zeitfenster, die sich für antiparasitäre Behandlungen eignen, mit den Zeiträumen zusammenfallen, in denen Dungorganismen inaktiv sind. Es wird jedoch empfohlen, die Häufigkeit antiparasitärer Behandlungen kritisch zu überprüfen und wenn möglich zu reduzieren. Soweit möglich sollten außerdem selektive oder gezielte, selektive Behandlungen anstelle von strategischen Behandlungen eingesetzt werden.

Um die Praktikabilität der RMM zu bewerten, werden umfassende Daten zum Zeitpunkt / der Häufigkeit der Verabreichung jedes Parasitizids in den verschiedenen Weidetierarten, -rassen und Altersklassen für die verschiedenen landwirtschaftlichen Methoden / Tierhaltungspraktiken benötigt. Einschränkungen der Zeiträume, in denen Parasitizide verabreicht werden, sollten für jede Nutztierart und Indikation in enger Zusammenarbeit mit Parasitologen festgelegt werden.

RMM: Das Produkt ist toxisch für Dungorganismen (Fliegen, Käfer). Deshalb dürfen Tiere nicht in aufeinanderfolgenden Jahreszeiten auf derselben Weide behandelt werden, um negative Auswirkungen auf die Dungfauna und ihre Prädatoren zu vermeiden

Durch diese Maßnahme können multivoltine Dungorganismen geschützt werden. Ob auch univoltine Arten von der RMM profitieren würden, hängt von der Überlappung ihres Reproduktionszyklus mit dem Zeitpunkt der antiparasitären Behandlung ab. Die Maßnahme erscheint grundsätzlich für alle drei betrachteten Weidetierarten praktikabel, wobei ihre Praktikabilität im Wesentlichen davon abhängt, dass ausreichend große Weideflächen für eine Wechselbeweidung zur Verfügung stehen. Ob und in welchem Umfang die RMM in die land-wirtschaftliche Praxis integriert werden kann, muss für jede Nutztierart und jedes landwirtschaftliche System ausgewertet werden. Bei der Spezifikation dieser RMM sollten auch andere Parasitizide mit derselben oder einer ähnlichen Wirkungsweise berücksichtigt werden. Es sollte also vermieden werden, Tiere in aufeinanderfolgenden Jahreszeiten auf derselben Weide mit Wirkstoffen zu behandeln, die dieselbe oder eine ähnliche Wirkungsweise haben.

RMM: Tiere in Freilandhaltung müssen während der Behandlung und während der nächsten X Tage nach der Behandlung im Stall gehalten werden

Dungorganismen würden von dieser RMM profitieren, wenn die Nutztiere für eine ausreichend lange Zeit eingestallt werden könnten. Die für einen Schutz der sensitivsten Dungorganismen (v.a. Sepsidae) notwendige Einstallungsdauer könnte jedoch sehr lang und infolgedessen nicht praktikabel sein. Eine Einstallung der Nutztiere während des Zeitraums der maximalen Exkretion der Parasitizide würde allerdings bereits zu einer deutlichen Reduktion der Exposition von Dungorganismen führen. Insgesamt ist die RMM in landwirtschaftlichen Betrieben praktikabel, in denen die Weidetiere nicht ganzjährig auf Weiden gehalten werden, wenn die notwendige Einstallungsdauer nicht zu lang ist und die Weiden relativ dicht an den Ställen liegen. Die RMM muss für jedes Antiparasitikum, jeden Verabreichungsweg, jede Dosis, Nutztierart und -rasse spezifiziert werden. Außerdem werden weitere Daten zur Ökologie von Dungorganismen (z.B. Dauer der Lebenszyklen, horizontale Verteilung) und zu ihrer Sensitivität gegenüber Antiparasitika benötigt, um die RMM ausreichend zu spezifizieren.

RMM: Dung/Gülle von behandelten Tieren ist vor dem Ausbringen auf landwirtschaftliche Flächen für mindestens X Tage/Monate zu lagern, um einen Abbau des Wirkstoffs zu ermöglichen

Bodenorganismen profitieren von dieser RMM, wenn der Dung bzw. die Gülle so lange gelagert werden kann, dass der Wirkstoff in ausreichendem Maße abgebaut wird. Die Maßnahme kann eingesetzt werden, wenn Dung bzw. Gülle vor der Ausbringung auf landwirtschaftliche Flächen gelagert wird. Dieser Dung / diese Gülle wird in meisten Fällen von (ständig oder zeitweise) eingestallten Tieren produziert. Daher ist die RMM für die Rinder- und Pferdehaltung relevant, i. Allg. jedoch nicht für Schafhaltung. Die Praktikabilität der Maßnahme hängt von der für den Wirkstoff notwendigen Lagerungsdauer in Dung bzw. Gülle der betreffenden Nutztierart ab. Die RMM muss für jeden Wirkstoff basierend auf seiner Halbwertszeit (DT_{50}) in Dung/Gülle der betreffenden Nutztierart spezifiziert werden. Für die im vorliegenden Projekt betrachteten Parasitizide fehlen jedoch öffentlich verfügbare Daten zur DT_{50} in Dung/Gülle.

RMM: Bei der Ausbringung von Dung/Gülle von behandelten Tieren auf landwirtschaftliche Flächen darf die beaufschlagte Menge an Gesamtstickstoff X (X<170) kg Stickstoff (N) je Hektar und Jahr nicht überschreiten

Durch diese Maßnahme können Bodenorganismen geschützt werden. Die RMM kann bei der Ausbringung von Dung bzw. Gülle auf landwirtschaftliche Flächen eingesetzt werde. Sie ist daher für die Rinder- und Pferdehaltung relevant, in dem meisten Fällen hingegen nicht für die Schafhaltung. Die RMM ist praktikabel, wenn ausreichend große landwirtschaftliche Flächen zur Verfügung stehen, auf die der Dung bzw. die Gülle ausgebracht werden kann. In Regionen mit intensiver Viehhaltung stehen solche Flächen u.U. nicht in ausreichendem Umfang zur Verfügung. Wenn überschüssiger Dung bzw. überschüssige Gülle abgegeben wird, muss sichergestellt werden, dass die Information zur Reduktion der maximal zu beaufschlagenden Menge an Gesamtstickstoff pro Hektar und Jahr von dem Landwirt, der den Dung/die Gülle abgibt, an den Landwirt, der den Dung/die Gülle ausbringt, weitergegeben wird. Die RMM muss für jedes Antiparasitika-Produkt, jede Nutztierart, Dosis, Verabreichungshäufigkeit und jedes Gülle-Ausbringungs-Szenario spezifiziert werden. Bei der Spezifikation dieser RMM sollten auch andere Parasitizide mit derselben oder einer ähnlichen Wirkungsweise berücksichtigt werden.

RMM: Dung/Gülle, der/die den Wirkstoff enthält, darf in aufeinanderfolgenden Jahren nicht auf dieselbe Fläche ausgebracht werden, um eine Akkumulation des Wirkstoffs zu verhindern

Mit dieser RMM kann die Akkumulation eines Parasitizids im Boden und damit auch die Exposition von Bodenorganismen reduziert werden. Um zu entscheiden, ob die RMM für ein Parasitizid implementiert werden muss, sollte eine konsistente Herangehensweise gewählt werden, die auf einer $DT_{50\ Boden}$ > 120 Tagen, einer $DT_{90\ Boden}$ > 1 Jahr und / oder einem $PEC_{Boden\ Plateau}$ zu PNEC-Verhältnis ≥ 1 basieren sollte. Die RMM kann angewandt werden, wenn Dung/Gülle auf landwirtschaftliche Flächen ausgebracht wird, und ist daher v.a. für die Rinder- und Pferdehaltung relevant. Die RMM ist praktikabel, wenn ausreichend große landwirtschaftliche Flächen, auf die der Dung bzw. die Gülle ausgebracht werden kann, zur Verfügung stehen. In Regionen mit intensiver Viehhaltung ist das u.U. problematisch. Wenn überschüssiger Dung/überschüssige Gülle abgegeben wird, muss sichergestellt werden, dass die Information zu dem für die Behandlung der Dung/Gülle-produzierenden Tiere verwendeten Parasitizid von dem Landwirt, der den Dung/die Gülle abgibt, an den Landwirt, der den Dung/die Gülle ausbringt, weitergegeben wird. Bei der Spezifikation dieser RMM sollten auch andere Parasitizide mit derselben oder einer ähnlichen Wirkungsweise berücksichtigt werden: Es sollte vermieden werden, in aufeinanderfolgenden Jahren auf dieselbe Fläche Dung/Gülle auszubringen, der/die Wirkstoffe mit derselben oder einer ähnlichen Wirkungsweise enthält.

Mögliche Anwendungsbeschränkungen für zugelassene Parasitizide

In Deutschland stehen insgesamt nur relativ wenige Parasitizid-Wirkstoffe für Weidetiere zur Verfügung und die Aussichten, dass in näherer Zukunft neue Wirkstoffe mit einer ähnlichen Wirksamkeit gegenüber den Zielorganismen, aber einer geringeren Toxizität für Dung- und Bodenorganismen entwickelt werden, sind begrenzt. Aufgrund dessen und wegen der aktuellen Resistenzsituation erscheinen Beschränkungen der Anwendung der zurzeit zugelassenen Avermectine und Milbemycine schwierig.

Zusammenfassung der Auswertung von Risikominderungsstrategien

Parasitizide sind gegenwärtig ein essentieller Bestandteil von Strategien zur Parasitenkontrolle in Weidetieren. Ihr umsichtiger Einsatz ist der vielversprechendste Ansatz, um negative Auswirkungen auf Dung- und Bodenorganismen zu reduzieren. Ein zentraler Punkt ist dabei die Minimierung des Einsatzes von Parasitiziden durch einen Ersatz strategischer Behandlungen durch selektive oder gezielte, selektive Behandlungen, soweit möglich.

Risikominderungsmaßnahmen können dazu beitragen, das Risiko für Dung- und Bodenorganismengemeinschaften zu reduzieren. Für die meisten der ausgewerteten RMM wurden jedoch Datenlücken

identifiziert, die gefüllt werden müssen, um die betreffende RMM ausreichend zu spezifizieren und anschließend ihre Effektivität und Praktikabilität vollständig bewerten zu können. Solche eine Bewertung muss für jedes Antiparasitika-Produkt und für jede Weidetierart durchgeführt werden. In diesem Zusammenhang soll angemerkt werden, dass auch Maßnahmen, die nur unter bestimmten Bedingungen angewendet werden können (z.B. abhängig von den Tierhaltungspraktiken und landwirtschaftlichen Methoden), dazu beitragen können, das durch Tierarzneimittel verursachte Risiko für die Umwelt deutlich zu reduzieren.

In Hinblick auf die Biologie und Ökologie von Dungorganismen, die Effekte von Parasitiziden auf Dungorganismengemeinschaften sowie die Weiterentwicklung von (a) nachhaltigen Herangehensweisen zur Kontrolle von Parasiten und (b) Risikominderungsminderungsmaßnahmen wurde Forschungsbedarf identifiziert.

Abschließend soll darauf hingewiesen werden, dass die aktuelle wirtschaftliche Situation von Landwirten ein wesentlicher Faktor ist, der die Praktikabilität etlicher Risikominderungsstrategien begrenzt.

1 Background and objective of the project

First studies on the effects of parasiticides on organisms in the environment have already been performed 40 years ago (Blume 1976). To date, a large number of veterinary pharmaceuticals including parasiticides have been detected in the environment (e.g. Halling-Sørensen et al. 1998, Boxall et al. 2002, 2006, Thiele-Bruhn 2003, Stamm et al. 2008, Bergmann et al. 2011, Iglesias et al. 2014).

In the authorisation process of veterinary medicinal products, the German Environment Agency (Umweltbundesamt, UBA) assesses the environmental risks. The impact assessment for the environment is described in specific guidelines (VICH 2000, 2005, EMEA/CVMP 2008). Data generated in authorisation processes and scientific research projects (e.g. the EU project ERAPharm) show that parasiticides such as avermectins (e.g. ivermectin) and milbemycins have a high toxicity to aquatic and terrestrial, especially dung-inhabiting, organisms (Floate et al. 2002, 2005, Garric et al. 2007, Liebig et al. 2010, Lumaret et al. 2012, Römbke et al. 2017, Tixier et al. 2016). In addition to the effects they can cause in the environment, many of these parasiticides are known to be persistent. Moreover, they might have a potential to bioaccumulate (see e.g. EMA/CVMP 2016a). Besides highly used antiparasitic substance ivermectin, other avermectins (e.g. doramectin and eprinomectin) as well as the milbemycin moxidectin are authorised in a range of products that can be used in food-producing species and horses (and, additionally, in non-food producing animal species).

However, even though the mentioned substances are already on the market for decades, a complete environmental risk assessment including a PBT assessment, i.e. an assessment of persistence (P), bioaccumulation (B) and toxicity (T), is not possible, because some data are lacking. The reason for this is that most of the antiparasitics can be found in products that were placed on the market before the environmental risk assessment was obligatory for the authorisation.

Therefore, the present project was initiated in 2014 in order to generate some of the missing data and to contribute to an overview of the environmental relevance of selected parasiticides regarding their potential to bioaccumulate and their toxicity to dung organisms.

Specifically, the project is contributing to filling data gaps by

a) deriving octanol/water partition coefficients for ivermectin and selamectin (section 3),
b) determining fish bioconcentration factors for ivermectin and doramectin (section 4),
c) compiling and evaluating data on the excretion of commercially available avermectins and milbemycins by pasture animals (section 7) and on the effects of these parasiticides on dung organisms (section 8).

In addition, the project contributes to a further evaluation of risk management strategies for parasiticides (avermectins and milbemycins) used in pasture animals (cattle, horses, sheep) by compiling and discussing

a) sustainable approaches to control parasites in pasture animals (section 9.3) and
b) risk mitigation measures aiming at the protection of dung (section 9.4.1) and soil organisms (section 9.4.2), taking the compiled data on excretion of the avermectins and milbemycins and on their toxicity to non-target organisms into account as far as possible (i.e. where detailed data for the respective active ingredients, application forms and pasture animal species were available).

The results of the project were discussed during a workshop ('Risk management strategies for parasiticides used in pasture animals', 18-19 January 2017) with representatives from competent authorities and industry, veterinarians and farmers. A workshop summary (in German) and a list of workshop participants are included in Annex 2 of this report. The outcome of this workshop was considered when preparing the present report.

2 Parasiticides considered in the present project

As mentioned in section 1, the experimental work within the project focuses on ivermectin, selamectin and doramectin (sections 3 and 4). In the theoretical work packages (sections 5–9), avermectins (doramectin, eprinomectin, ivermectin) and milbemycins (moxidectin) that are commercially available in Germany are considered. A list of these compounds was provided by UBA. This list also includes some compounds, which are not authorised for use in pasture animals (avermectin B_1, emamectin, selamectin, milbemycinoxim), but might be possible alternatives to the authorised substances. Table 1 provides an overview of the considered parasiticides and their routes of administration in pasture animals (cattle, sheep, and horses).

Table 1: Avermectins and milbemycins considered in the present project: overview of routes of administration and treated livestock animals for products, which are authorised in Germany

Active pharmaceutical ingredient	Route of administration						Treated livestock animal species		
	Injection	Pour-on	Oral (paste)	Oral (gel)	Oral (tablet)	Oral (solution)	Cattle	Sheep	Horses
Avermectins									
Doramectin	x						x	x	
		x					x		
Eprinomectin		x					x		
Ivermectin	x						x	x	
		x					x		
			x						x
				x					x
					x				x
Avermectin B_1 (abamectin)	Only authorised as plant protection product								
Emamectin	Only authorised for use in fish								
Selamectin	Only authorised for use in cats and dogs								
Milbemycins									
Moxidectin	x						x		
		x					x		
				x					x
						x		x	
Milbemycinoxim	Only authorised for use in cats and dogs								

If livestock animals are treated on the pasture or in the stable depends on the parasite (see e.g. Liebisch et al. 2002) and, partly, on practical considerations (farm management, work load, personal resources; see also section 9.3.1).

3 Laboratory tests to determine the octanol/water partition coefficients of ivermectin and selamectin

The equilibrium distribution of a chemical between two phases, which are immiscible to a high extend, can be described by the partition coefficient. The partition coefficient between water and 1-octanol (P_{OW}) is defined as the ratio of the equilibrium concentrations of the test substance in 1-octanol saturated with water and water saturated with 1-octanol (OECD 2006). Quite often, P_{OW} is indicated as log P_{OW}. The P_{OW} (or log P_{OW}) values provide preliminary estimates of mobility, transport and bioaccumulation and are needed as input data for environmental modelling.

Standardised methods for the determination of 1-octanol/water partition coefficients are described in the OECD test guidelines (TG) 107 (OECD 1995), 117 (OECD 2004) and 123 (OECD 2006). Due to the transfer of octanol micro-droplets into the water phase, the shake flask method (OECD TG 107) might yield artefacts for substances with expected high P_{OW} values leading to an overestimation of the substance concentration in the water and an underestimation of the P_{OW} value. Therefore, the use of this method is not recommended for substances with expected log P_{OW} values >4. Artefacts associated with the shake flask method can be reduced by using the slow stirring method as described in OECD TG 123, which is appropriate for substance with log P_{OW} > 4.

A review of publicly available literature shows that a number of citations is available for the log P_{OW} of ivermectin (e.g. Bloom & Matheson et al. 1993, Oppel et al. 2004, Rath et al. 2016). However, they refer to the same test result published by Halley et al. (1989c). In this publication, a P_{OW} of 1651 (corresponding to a log P_{OW} of 3.22) is indicated, which is cited as 'S.H.L. Chiu and R. Sestokas, personal communication'. The P_{OW} of 1651 is also mentioned in US dossiers (e.g. US FDA 1990), however without details on the experimental methods (see also Liebig et al. 2010). As OECD TG 123 was published in 2006, it is obvious that the test was not performed using the slow-stirring method and that the log P_{OW} might be underestimated. This assumption is supported by the result of a QSAR estimation using EPIsuite, log Kow (version 1.68 estimate, KowWin 2010), a log P_{OW}-value of 4.11.

For selamectin, information on the log P_{OW} was neither available from publicly accessible literature nor by EPIsuite calculation performed by Fh-IME. Expert judgement leads to the assumption that selamectin is likely to have a log P_{OW} value similar to that estimated for ivermectin.

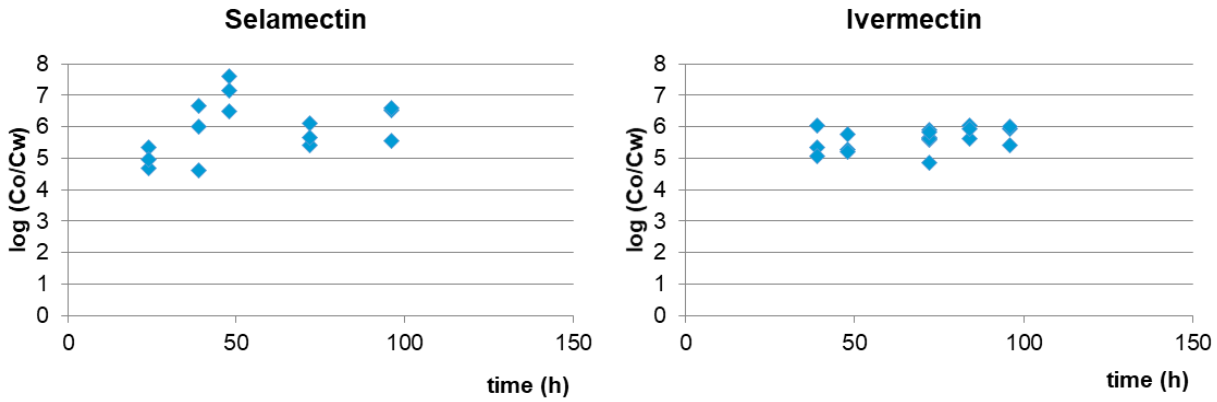
Since it was expected that the log P_{OW} values of selamectin and ivermectin are > 4, the slow stirring method (OECD TG 123) was applied.

The experimental tests were performed by the Fraunhofer-Institute for Molecular Biology and Applied Ecology (Fh-IME) in brown glass bottles (250 mL), which were filled with 100 mL of water and 20 mL of 1-octanol spiked with the test substance. The glass bottles and the further equipment were thermostated in an incubator at a temperature of 25°C; slow-stirring was performed using teflon-coated magnetic stir bars.

In a pilot slow-stirring experiment, the length of the equilibration period was determined by consecutive sampling of the water and the 1-octanol phase and subsequent determination of the test item in both phases. It could be shown that the ratio of the concentrations of the test substance in 1-octanol and water (C_O/C_W ratio) was in equilibrium already after 24 h for selamectin and after 39 h for ivermectin.

The P_{OW} values and the corresponding average log P_{OW} values (log $P_{OW, Av}$) were determined in three slow-stirring experiments under identical conditions. To demonstrate that an equilibrium was reached, the C_O/C_W ratio was determined at five consecutive time points (see Figure 1). These values were used for deriving the log $P_{OW, Av}$. Furthermore, multiple sampling allowed calculating the standard error σ of the log $P_{OW, Av}$.

Figure 1: Ratio of the concentrations in 1-octanol and water (C_O/C_W) for selamectin and ivermectin measured at five consecutive time points during equilibrium.



Own presentation, Fraunhofer-Institute for Molecular Biology and Applied Ecology.

The average 1-octanol/water partition coefficients expressed as log P_{OW} values of the test items determined at a temperature of 25.0°C with their standard error σ log $P_{OW, Av}$ were 6.0 ± 0.7 for selamectin and 5.6 ± 0.3 for ivermectin. The obtained standard deviations are rather low for such a study type and indicate reliability of the results.

From these results obtained with the slow-stirring method it can be concluded that the so far published log P_{OW} of 3.22 (Halley et al. 1989c, US FDA 1990) underestimates the log P_{OW} of ivermectin, possibly because of methodological drawbacks when using the shake-flask method. This might also be true for the QSAR-estimation using EPIsuite which is probably based mainly on results obtained with the shake-flask method. Thus, OECD TG 123 is supposed to be the method of choice for the highly lipophilic compounds ivermectin and selamectin.

Furthermore, it is concluded that – although the slow-stirring method is much more complex and sophisticated than the shake-flask method – it should be applied in all cases where a log P_{OW} around 4 is to be expected. The method is also recommended for substances with an estimated log P_{OW} slightly below 4 to avoid an underestimation of the log P_{OW}.

An exact description of the two laboratory tests is provided in study reports that were submitted to the UBA (Herrchen 2015 a, b).

For many substances, log P_{OW} values and bioconcentration factors are correlated (see e.g. Arnot & Gobas 2006). In EMEA/CVMP (2008), equations are indicated that can be used to estimate the bioconcentration factors based on the log K_{OW} for substances with (a) log P_{OW} values between 2 and 6 and (b) log P_{OW} values above 6. However, it is noted that a molecular weight >700 g/mol often leads to a lower bioconcentration than estimated when using these equations. Both ivermectin (875 g/mol) and selamectin (770 g/mol) have such a high molecular weight. In addition, a large substance diameter and active efflux mechanisms can reduce bioconcentration (de Wolf et al. 2007, Arnot et al. 2009, Schlechtriem et al. 2015).

If the active substance of a veterinary medicinal product has a log P_{OW} values ≥4, absorption, distribution, metabolism and excretion data, as well as information from biodegradation studies and molecular weight shall be considered to verify if the substance has the potential to bioaccumulate (VICH 2005). If this is the case, a bioconcentration study shall be carried out (see section 4).

4 Laboratory tests to determine bioconcentration of ivermectin and doramectin in fish

The bioconcentration factors of ivermectin and doramectin were determined by ECT according to OECD test guideline 305 (OECD 2012) using zebrafish (*Danio rerio*) as test organism. Differential chemical analysis of the test substances and potential metabolites in fish tissue and water was performed by the University of Gießen.

4.1 Available data on bioconcentration and fish toxicity of ivermectin and doramectin

A literature search using Scopus was performed by ECT to identify data on bioconcentration and fish toxicity of ivermectin and doramectin that are relevant for planning the bioconcentration tests. Given that Scopus primarily contains peer-reviewed publications, an additional Google search was carried out to identify non peer-reviewed literature (e.g. reports, dossiers).

No data on bioconcentration in fish were identified for ivermectin and doramectin. Available studies on the uptake of ivermectin by fish mainly focus on the tissue distribution (e.g. Høy et al. 1992) and on depuration times after oral application to derive withdrawal periods (e.g. Roth et al. 1993). In mussels (*Mytilus edulis*), a bioconcentration factor (BCF) of 750 on wet weight basis was determined after 6 d exposure to 6.9 µg/L of ivermectin. In this study, steady state was not reached (Davies et al. 1997; see Annex 1, Table 34). Bioconcentration studies with the related substance avermectin B_1 indicate a relatively low bioconcentration in fish with bioconcentration factors ranging from 41 to 56 on a wet weight basis (Wislocki et al. 1989, Van den Heuvel et al. 1996, Shen et al. 2005; cf. Annex 1, Table 34). Steric hindrance of uptake of the large molecule was assumed responsible for the low accumulation (Van den Heuvel et al. 1996). However, according to information from the UBA a lipid-normalised BCF of > 2000 was derived in a recent fish bioconcentration study with moxidectin, resulting in the classification of moxidectin as bioaccumulative (see also EMA/CVMP 2016a).

In bioconcentration tests, fish are exposed to substance concentrations that are below chronically toxic levels. Both ivermectin and doramectin are highly toxic to fish. Acute fish toxicity data (96-h LC_{50} values) for ivermectin range from 3.0 to 73 µg/L (Halley et al. 1989a, b, Kilmartin et al. 1996, Domingues et al. 2016, B. Halling-Sørensen, pers. comm.; see Annex 1, Table 35), and from 5.1 to 11 µg/L for doramectin (US FDA 2002; Annex 1, Table 35). All LC_{50} values are based on nominal concentrations. In a recent 21-d study, nominal ivermectin concentrations ≥ 0.25 µg/L were shown to affect the swimming behaviour of zebrafish (*Danio rerio*), while growth was reduced at ≥ 2.5 µg/L in male and at 25 µg/L in female fish. At the highest tested concentration (25 µg/L), fish were lethargic (Domingues et al. 2016). Neither for ivermectin nor for doramectin further chronic fish toxicity data were identified that are relevant for the project (i.e. relate to aqueous exposure).

4.2 Differential analysis of ^{3}H-ivermectin und ^{3}H-doramectin

Differential analysis of ^{3}H-ivermectin und ^{3}H-doramectin was established at the University of Gießen as described in the following.

Extraction and recovery of ivermectin, doramectin, avermectin B_1 and moxidectin from fish samples

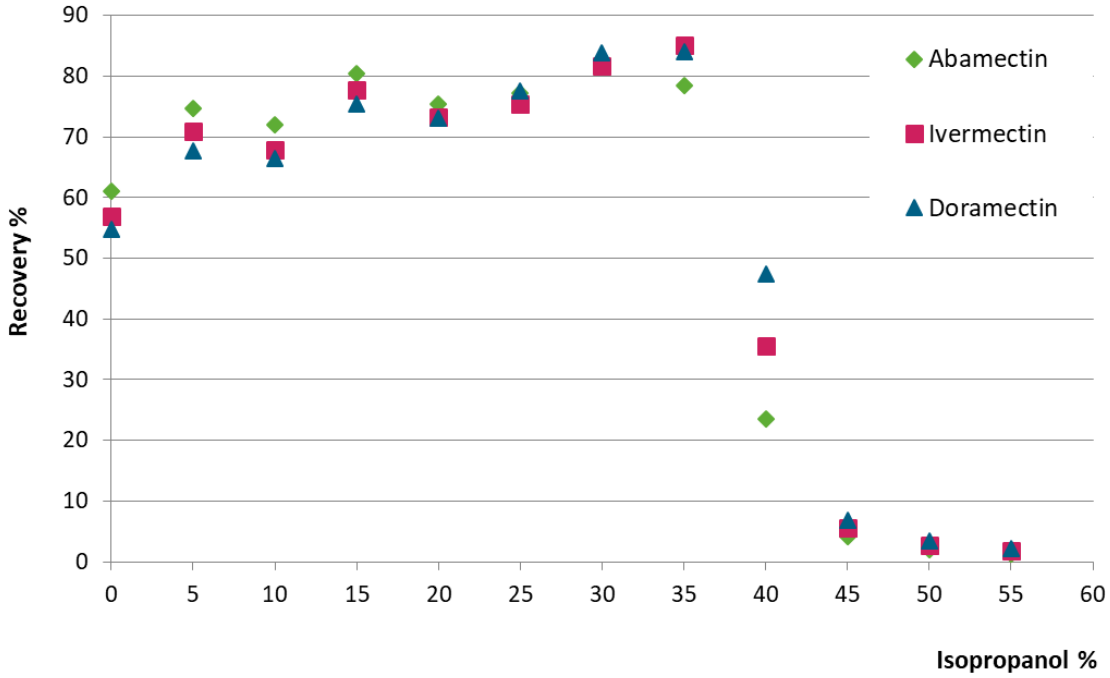
Recovery tests with spiked fish samples resulted in an optimised solvent mixture of 5% isopropanol and 95% acetonitrile. Four fish samples were spiked with moxidectin, avermectin B_1, doramectin and ivermectin (two samples with 0.05 µg/sample and 0.25 µg/sample, respectively). The macrocyclic lactones moxidectin and avermectin B_1 (abamectin) were included in the recovery studies, as they show a C18-retention and sorption behaviour similar to the expected metabolites of ivermectin and

doramectin. Fish samples were homogenized with an ultra turrax blender in 15 mL centrifuge tubes. Subsequently, 0.5 mL isopropanol was added, and the mixture was homogenised again with addition of 9.5 mL acetonitrile. The initial addition of isopropanol minimised precipitation of the protein rich fish matrix in acetonitrile. After additional stirring and sonication, the mixture was centrifuged. Following concentration and derivatisation of the supernatant, moxidectin, avermectin B_1, doramectin, and ivermectin were determined by high performance liquid chromatography (HPLC) with fluorescence detection. After extraction of spiked fish matrix, mean recovery rates of 81%, 76%, 77%, and 80% were obtained for moxidectin, avermectin B_1, doramectin and ivermectin, respectively.

Extraction and recovery of ivermectin, doramectin and avermectin B_1 from water

For extraction and clean-up of ivermectin, doramectin and avermectin B_1, C18 solid phase extraction was used. After conditioning of the cartridges with isopropanol and a mixture of isopropanol and water, water samples were pumped through the sorbent matrix and subsequently eluted. Different isopropanol contents were evaluated. Extraction was optimised by using 20% isopropanol. Recovery rates between 75 and 80% were obtained (Figure 2).

Figure 2: Recovery (%) of ivermectin, doramectin and avermectin B_1 (abamectin) depending on the isopropanol fraction used to pre-condition the C18 cartridges.



Own presentation, Justus Liebig University.

Method development for differential analysis of radiolabelled avermectins

The following analytical methods were set-up for the bioconcentration studies. Extracts were analysed using (1) a sophisticated thin layer chromatographic (TLC) method, and (2) reversed-phase HPLC (RP-HPLC) with subsequent fractionation. Both methods allow for a separation of the parent compound (ivermectin, doramectin) from expected metabolites. This was tested using specifically synthesized metabolites of ivermectin (ivermectin monosaccharide and ivermectin aglycone).

Radioactivity after TLC can be detected very sensitively using a phosphor imager. Two options to detect the radioactivity after HPLC were available: (a) portions of the isolated fractions could be spotted

onto TLC plates for subsequent determination with the phosphor imager, (b) aliquots of the fractions could be analysed by liquid scintillation counting (LSC). Both methods allow the detection of low activities (TLC: down to 5 Bq per sample, LSC: down to 1 Bq per sample).

After evaluating the feasibility and sensitivity of these two methods, it was decided to work with HPLC separation and LSC detection for the definitive bioconcentration tests with ivermectin and doramectin.

4.3 Bioconcentration of ^{3}H-ivermectin in zebrafish

The bioconcentration factor (BCF) of ivermectin in zebrafish (*Danio rerio*) was determined by ECT according to OECD test guideline 305 (OECD 2012). A pre-test was carried out with two ivermectin concentrations (0.1 and 1.0 µg/L). Since this test did not demonstrate a clear test concentration independence of the accumulation, the definitive test was also performed using two concentrations. However, lower ivermectin concentrations (0.01 µg/L and 0.10 µg/L, both corresponding to 800 dpm/mL) were used to avoid the risk of sublethal effects on fish. The definitive test included a solvent control and a water-only control. The uptake phase was terminated after 20 d of exposure; the subsequent depuration phase lasted 10 d. Fish and water samples were taken as described in Table 2.

Table 2: Sampling schedule for the definitive test with ivermectin

	Ivermectin concentration	
	0.01 µg/L	**0.10 µg/L**
Uptake phase		
TRR Fish	d 1, 3, 6, 8, 10, 14, 15, 17 (4 fish each), d 20 (6 fish each)	
TRR Water	daily (at least 2 samples)	
PRP Fish	d 20 (10 fish)	d 3, 6, 8 (10 fish each), d 20 (20 fish)
PRP Water	d 17 und 20 (1-2 samples)	d 3, 6 ,8, 17, 20 (1-2 samples)
Depuration phase		
TRR Fish	d 0.21, 2, 4, 7 (4 fish each), d 10 (6 fish each)	
TRR Water	on fish-sampling days	

Abbreviations: d = day after start of exposure (uptake phase) and day after start of depuration phase, PRP = percentage of radioactivity associated with the parent compound, TRR = total radioactive residues

In the fish exposed to ivermectin, no behavioural differences from the control fish were observed. In all treatments and controls, no mortality was recorded.

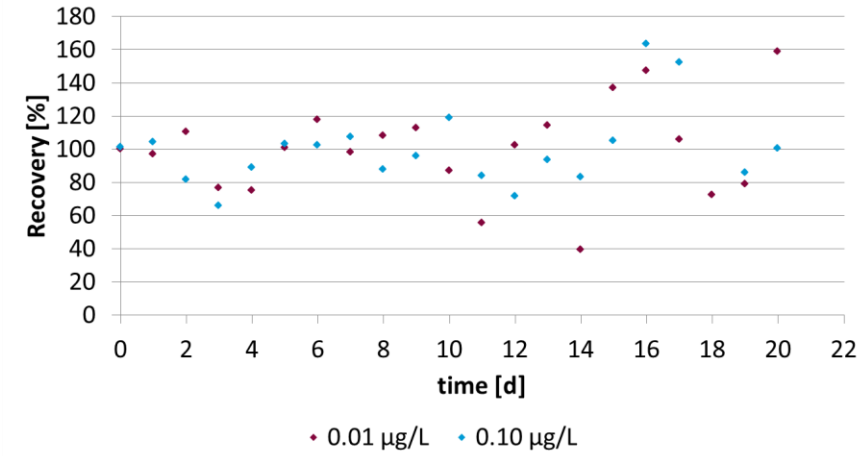
The mean recovery of the ivermectin concentration in water per sampling date was in the range of 40 to 159% (0.01 µg/L) and 66 to 163% (0.10 µg/L) of the mean measured concentration (0.01 µg/L: 788 dpm/mL; 0.10 µg/L: 868 dpm/mL). Most samples were in a range of ± 20% of the mean measured concentration (16 out of 20 sampling dates; see Figure 3).

At 0.01 µg/L, both the course of the concentrations in fish (C_f) and the accumulation factors (AFs) remained parallel to the x-axis after 6 days, except for days 14 and 15 (Figures 4 and 5). On these two sampling days, a temporary increase of accumulation was observed (Figure 5). However, concentrations in fish on day 10, the sampling date before day 14, did not show any significant difference from C_f at the two final sampling dates of the uptake phase (days 17 and 20). It is therefore considered that a statistically verified steady state was reached.

At 0.10 µg/L, the C_f and AF varied strongly until day 10 (Figures 4 and 5). After day 10, both the C_f and AF did not further increase, i.e. steady state was reached.

The time to reach 95% of steady state was 7.6 d at 0.01 µg/L and 0.2 d at 0.10 µg/L as calculated according to OECD test guideline 305 (OECD 2012).

Figure 3: Measured ivermectin concentrations in the water during the definitive test based on measured radioactivity expressed as % of mean measured radioactivity (788 dpm/mL at 0.01 µg/L, and 868 dpm/mL at 0.10 µg/L; n = 2 - 6).



Own presentation, ECT Oekotoxikologie GmbH.

Figure 4: Measured concentrations of ivermectin in fish (C_f in dpm/g fish wet weight) during the uptake phase of the definitive test at ivermectin concentrations of 0.01 µg/L and 0.10 µg/L (n = 4 for days 1-17, n = 6 for day 20 for both concentrations).

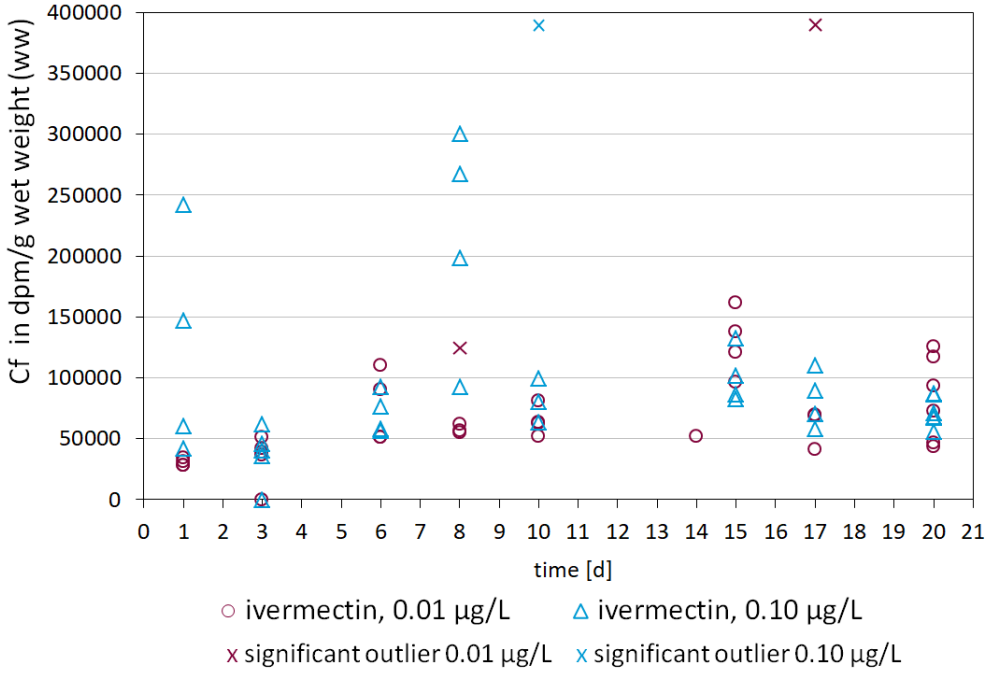


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Figure 5: Individual and mean accumulation factors (AF = concentration in fish (C_{fish}) / concentration in water (C_{water})) with standard deviations in the definitive test at ivermectin concentrations of 0.01 µg/L and 0.10 µg/L without significant outliers. n = 4 for days 1–17 except for days 8 and 10 (n = 3 for 0.10 µg/L) and day 17 (n = 3 for 0.01 µg/L), n = 6 for day 20 (both concentrations).

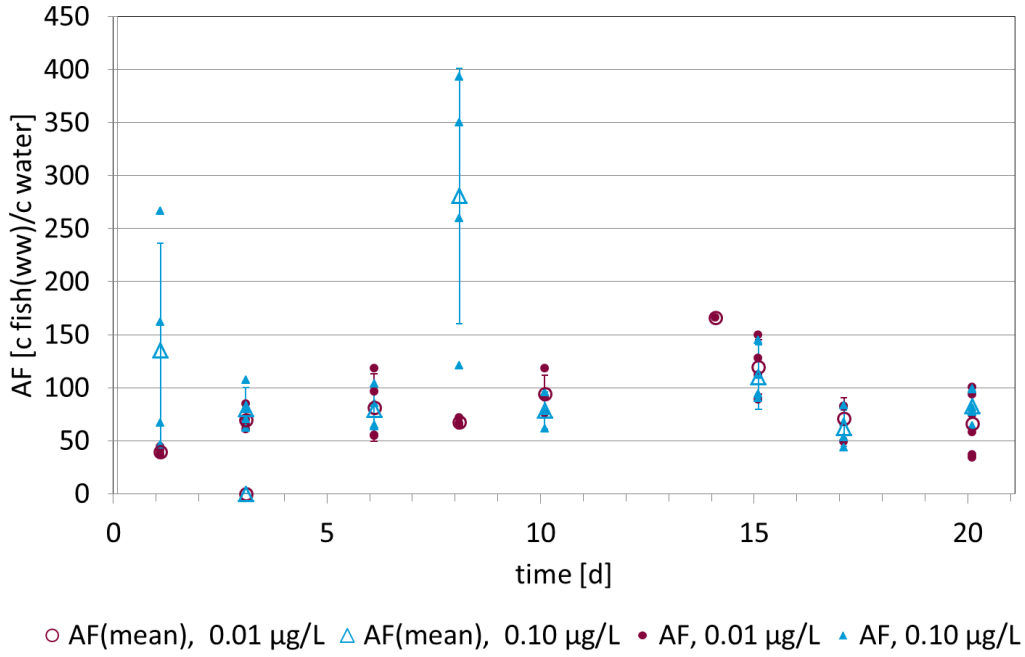

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Figure 6: Ivermectin uptake kinetics at 0.01 µg/L (nonlinear regression analysis). The accumulation factors (AF = concentration in fish (C_f) / concentration in water (C_w)) are based on total wet-weight-based concentrations in fish and mean radioactive concentrations in water at the corresponding sampling date.

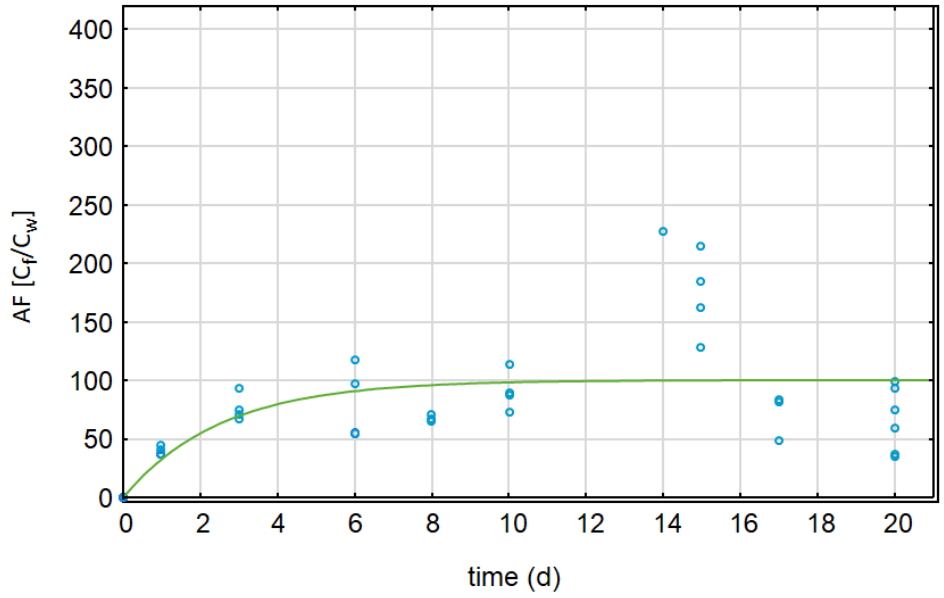

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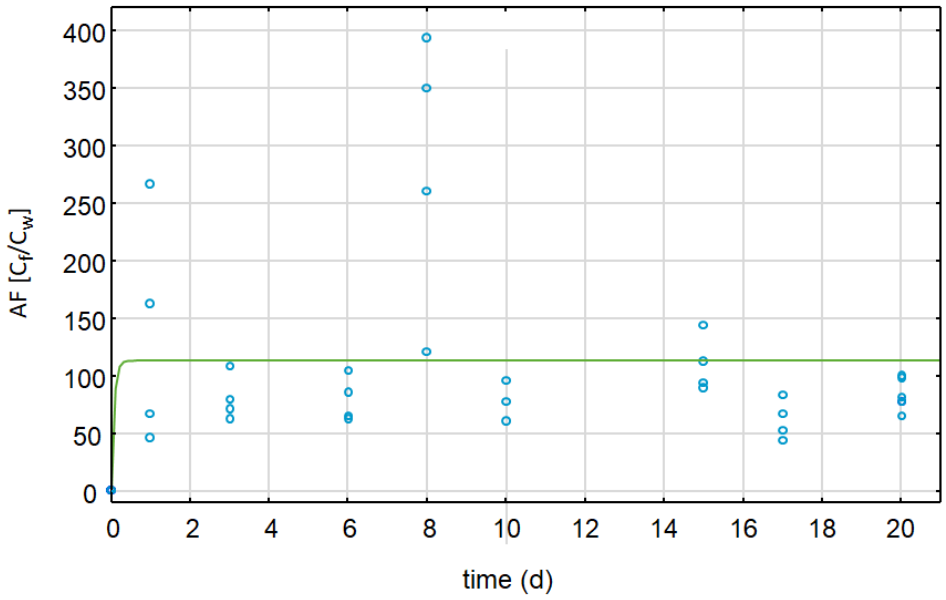
Figure 7: Ivermectin uptake kinetics at 0.10 µg/L (nonlinear regression analysis). The accumulation factors (AF) are based on total wet-weight-based concentrations in fish (C_f) and mean radioactive concentrations in water (C_w) at the corresponding sampling date.



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Kinetic bioconcentration factors (BCF_K) of 101 L/kg for 0.01 µg/L and 113 L/kg for 0.10 µg/L were calculated by nonlinear regression analysis using the accumulation factors throughout the uptake phase. Due to varying ivermectin concentrations in water, the calculation was based on the accumulation factors for individual fish (C_f / mean daily C_w, Tables 3 and 4). A concentration dependency of the accumulation was excluded because of the similar BCF values at both ivermectin concentrations. The determined BCF values (Tables 3 and 4) are much lower than the threshold value defined for the B-criterion in Annex XIII of the REACH regulation (BCF ≥ 2000 L/kg; EC 2011).

The estimated times for depuration of 50% of the accumulated ivermectin were 0.7 days at 0.01 µg/L and 1.5 days at 0.10 µg/L. The estimated times for depuration of 95% of the accumulated ivermectin were 3 days at 0.01 µg/L and 6.5 days at 0.10 µg/L (for the depuration kinetics, see Figures 8 and 9).

At 0.10 µg/L, the mean measured non-depurated residues were 0% of the accumulated radioactivity on days 24 and 27 (days 4 and 7 of depuration), but 33% for the last sampling date. The reasons for this increase of body residues at test end remain speculative. The increase might be explained with high concentrations in individual fish during the uptake phase. For individual fish having accumulated higher residues than others, as shown by relatively high inter-replicate variability of residues, the depuration phase might have been too short to warrant a complete elimination of the body residues. However, technical reasons (e.g. analytical carryover or accidental switch of samples) could be excluded.

Figure 8. Depuration kinetics for ivermectin (0.01 µg/L) derived using nonlinear regression analysis based on radioactive concentrations (dpm/g) of ivermectin in fish wet weight (C_f) throughout the depuration phase.

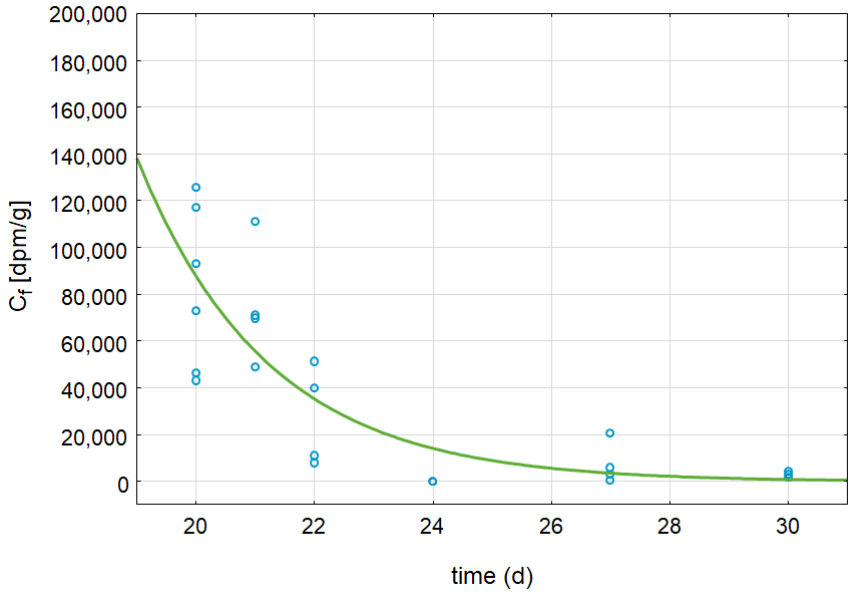

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Figure 9. Depuration kinetics for ivermectin (0.10 µg/L) derived using nonlinear regression analysis based on radioactive concentrations (dpm/g) of ivermectin in fish wet weight (C_f) throughout the depuration phase. With regard to the increased measured non-depurated residues on day 30, please see the discussion on the previous page.

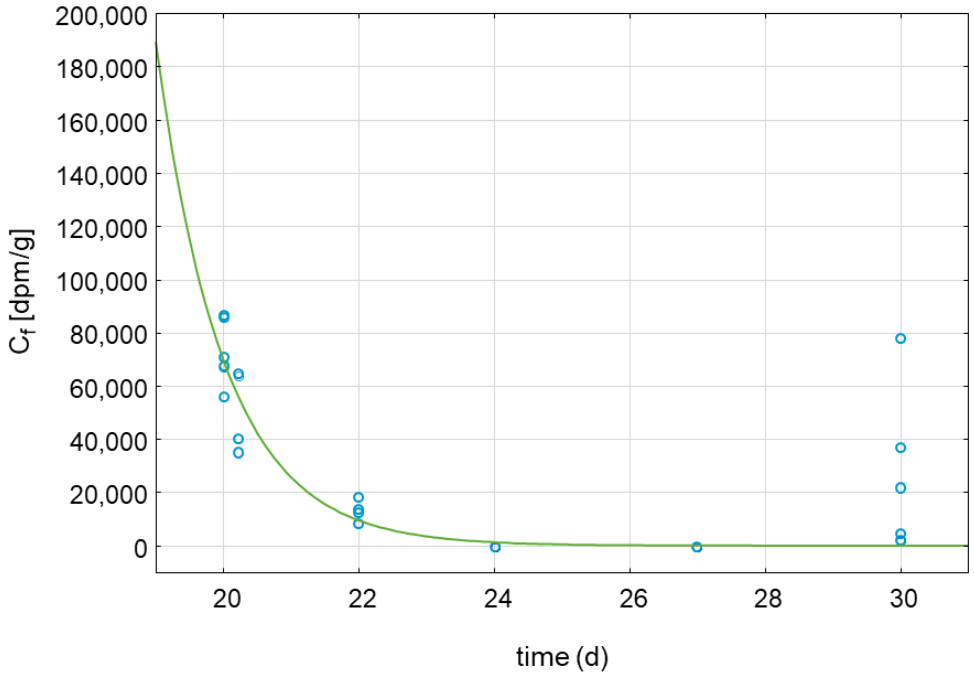

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Table 3: Summary of bioconcentration parameters for the lower ivermectin concentration (0.01 µg/L) (uptake phase). All data are based on accumulation factors (wet-weight-based). All bioconcentration factors related to parent compound (BCF_{SSpc}, BCF_{Kpc}, BCF_{SSLpc}, BCF_{KLpc} and BCF_{KgLpc}) are based on the results of the differential chemical analysis that are described in the see next section (Table 5).

Parameters	Estimate	Lower asymptotic 95% confidence interval	Upper asymptotic 95% confidence interval	R / R^2
BCF_{SS}	68 ± 24 L/kg	–	–	–
BCF_{K}	101 L/kg	95.8	1837	0.62 / 0.39
k_1	-39.9 L kg^{-1} d^{-1}	-76	-4.06	
k_2	-0.397 d^{-1}	-0.790	-0.00221	
Time to reach 95% steady state	7.6 d	–	–	–
BCF_{SSL}	63 L/kg	–	–	–
BCF_{KL}	99 L/kg	–	–	–
BCF_{Kg}	103 L/kg	–	–	0.42 / 0.18
k_g	-0.00764 d^{-1}	–	–	–
k_{2g}	-0.389 d^{-1}	–	–	–
BCF_{KgL}	101 L/kg	–	–	–
BCF_{SSpc}	64.3 L/kg	–	–	–
BCF_{Kpc}	95.4 L/kg	–	–	–
BCF_{SSLpc}	59.5 L/kg	–	–	–
BCF_{KLpc}	93.6 L/kg	–	–	–
BCF_{KgLpc}	93.6 L/kg	–	–	–

Abbreviations: BCF_K = kinetic bioconcentration factor, BCF_{Kg} = growth-corrected kinetic bioconcentration factor, BCF_{KgL} = lipid-normalised growth-corrected kinetic bioconcentration factor, BCF_{KgLpc} = lipid-normalised and growth-corrected kinetic BCF of parent compound, BCF_{KL} = lipid-normalised kinetic bioconcentration factor, BCF_{KLpc} = lipid-normalised kinetic bioconcentration factor (BCF_K) of parent compound, BCF_{Kpc} = kinetic bioconcentration factor (BCF_K) of parent compound, BCF_{SS} = bioconcentration factor at steady state, BCF_{SSL} = lipid normalised bioconcentration factor at steady state, BCF_{SSLpc} = lipid-normalised steady-state bioconcentration factor (BCF_{ss}) of parent compound, BCF_{SSpc} = steady-state bioconcentration factor (BCF) of parent compound, k_1 = k_u = uptake rate constant, k_2 = k_e = depuration rate constant, k_{2g} = growth-corrected depuration rate constant, k_g = growth rate constant, R = coefficient of correlation, R^2 = coefficient of determination.

Table 4: Summary of bioconcentration parameters for the higher ivermectin concentration (0.10 µg/L) (uptake phase). All data are based on accumulation factors (wet-weight-based). All bioconcentration factors related to parent compound (BCF_{SSpc}, BCF_{Kpc}, BCF_{SSLpc}, BCF_{KLpc} and BCF_{KgLpc}) are based on the results of the differential chemical analysis that are described in the see next section (Table 5).

Parameters	Estimate	Lower asymptotic 95% confidence interval	Upper asymptotic 95% confidence interval	R / R^2
BCF_{SS}	84 ± 24 L/kg	-	-	-
BCF_K	113 L/kg	72	129	0.41 / 0.17
k_1	- 1640 L kg^{-1} d^{-1}	-42,033,968	42,030,689	
k_2	- 14.5 d^{-1}	-372,443	372,414	
Time to reach 95% steady state	0.2 d	-	-	-
BCF_{SSL}	82 L/kg	-	-	-
BCF_{KL}	111 L/kg	-	-	-
BCF_{Kg}	113 L/kg	-	-	0.22 / 0.046
k_g	-0.00365 d^{-1}			
k_{2g}	-14.5 d^{-1}	-	-	
BCF_{KgL}	111 L/kg	-	-	-
BCF_{SSpc}	79.4 L/kg	-	-	-
BCF_{Kpc}	106.8 L/kg	-	-	-
BCF_{SSLpc}	77.5 L/kg	-	-	-
BCF_{KLpc}	104.9 L/kg	-	-	-
BCF_{KgLpc}	104.9 L/kg	-	-	-

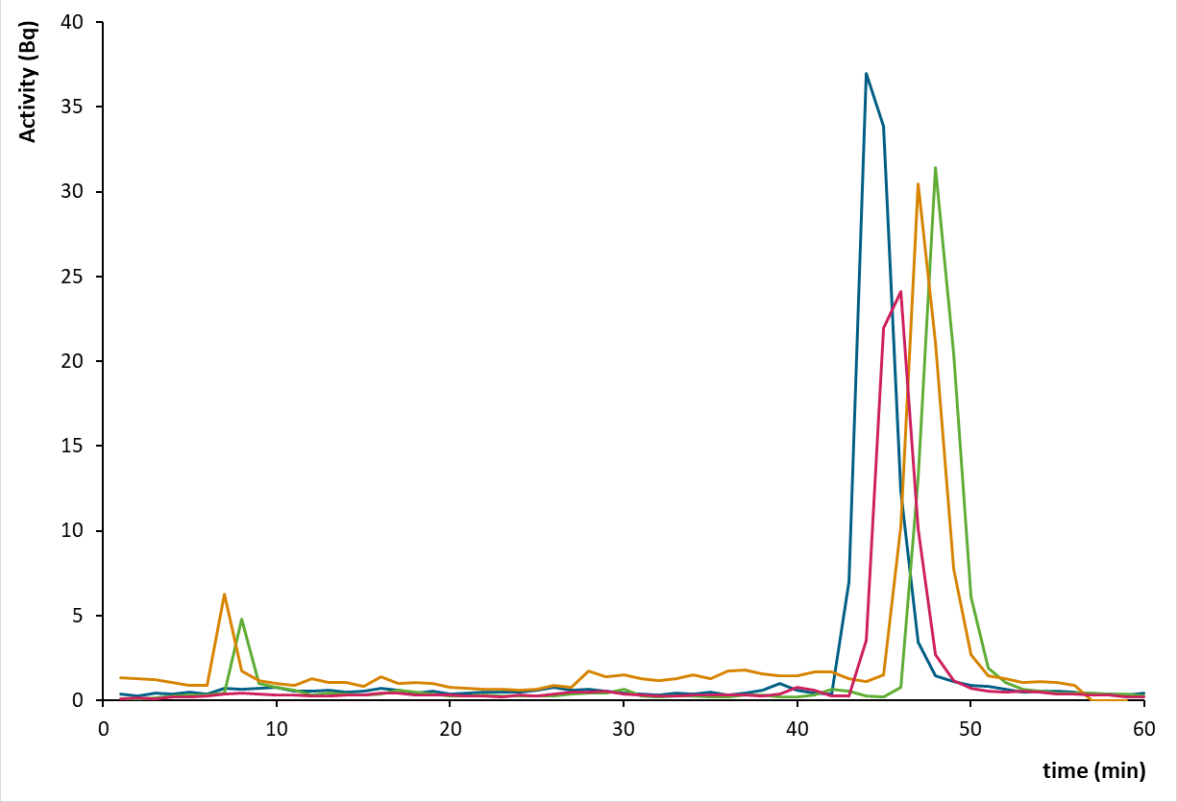
Abbreviations: BCF_K = kinetic bioconcentration factor, BCF_{Kg} = growth-corrected kinetic bioconcentration factor, BCF_{KgL} = lipid-normalised growth-corrected kinetic bioconcentration factor, BCF_{KgLpc} = lipid-normalised and growth-corrected kinetic BCF of parent compound, BCF_{KL} = lipid-normalised kinetic bioconcentration factor, BCF_{KLpc} = lipid-normalised kinetic bioconcentration factor (BCF_K) of parent compound, BCF_{Kpc} = kinetic bioconcentration factor (BCF_K) of parent compound, BCF_{SS} = bioconcentration factor at steady state, BCF_{SSL} = lipid normalised bioconcentration factor at steady state, BCF_{SSLpc} = lipid-normalised steady-state bioconcentration factor (BCF_{ss}) of parent compound, BCF_{SSpc} = steady-state bioconcentration factor (BCF_{ss}) of parent compound, k_1 = k_u = uptake rate constant, k_2 = k_e = depuration rate constant, k_{2g} = growth-corrected depuration rate constant, k_g = growth rate constant, R = coefficient of correlation, R^2 = coefficient of determination.

Results of the differential chemical analysis

At the end of the uptake phase, four water samples (500 mL) were taken and 120 mL isopropanol were added to each of the samples before solid-phase extraction. Four fish samples were extracted with acetonitrile and isopropanol. Chromatograms were obtained after fractionated HPLC and LSC-detection.

For the water samples, 100% of the measured radioactivity relate to the parent compound, i.e. ivermectin (Figure 10, Table 5). Signals obtained between minutes 5 and 10 were not reproducible.

Figure 10: Combined radio-chromatograms of the water extracts from the definitive bioconcentration test with ivermectin



Own presentation, Justus Liebig University.

For the fish samples, two additional signals with a combined average area of 5.5% were found at minutes 21 and 28 (Figure 11, Table 5). Comparing retention times to those of the reference compounds, these signals were identified as ivermectin monosaccharide and ivermectin aglycone. Further signals around minutes 10 and 35–40 were not reproducible.

Figure 11: Combined radio-chromatograms of the fish extracts from the definitive bioconcentration test with ivermectin

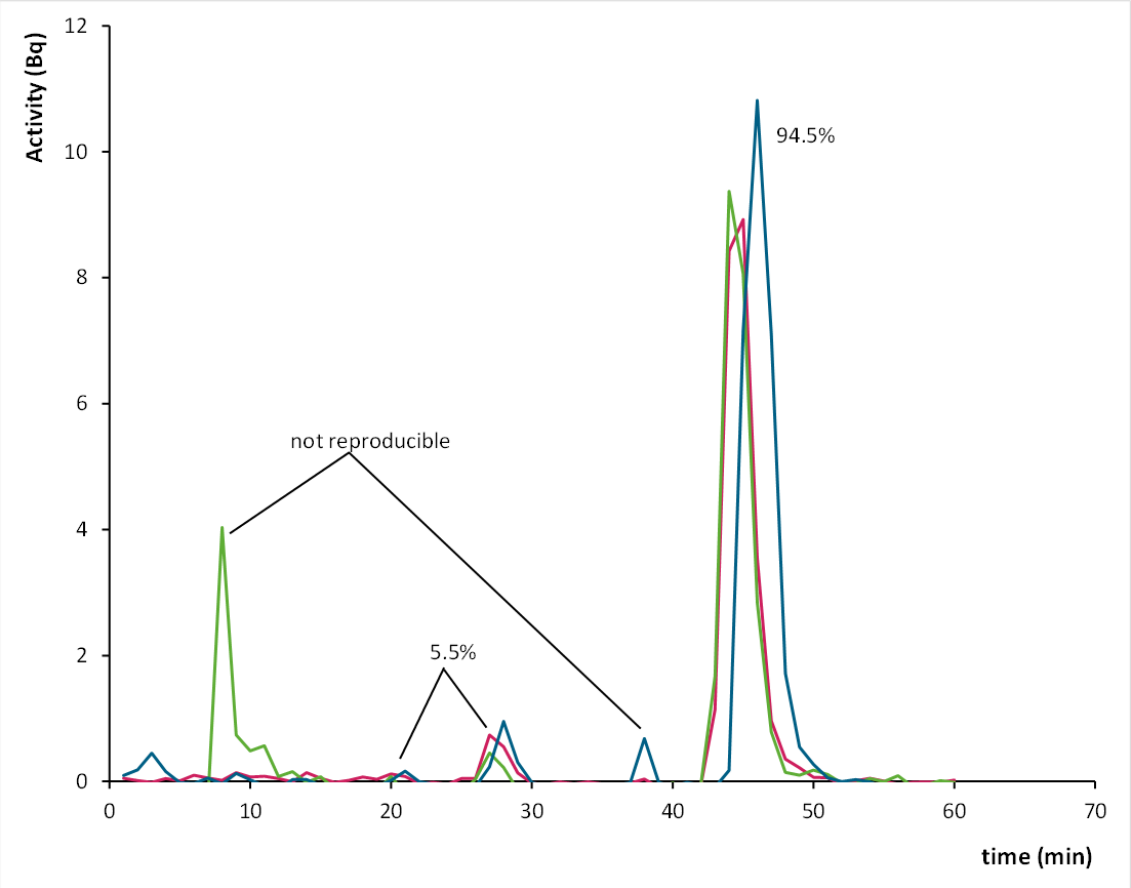


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Table 5: Overview of the mean percentages of parent compound and metabolites detected with specific chemical analysis in the definitive bioconcentration test with ivermectin

	Parent compound	Further signals (metabolites)
Water samples	100%	0%
Fish samples	94.5%	5.5%

Detection of ivermectin accumulation in different fish tissues

Thin sections (20 and 40 µm) of ivermectin-exposed fish may provide additional information on the distribution of the test substance in the fish tissue. Therefore, the preparation of thin sections was successfully established at the University of Gießen. Thin sections were prepared from three zebrafish sampled at the end of the definitive test with ivermectin. Radioactivity was detected using a phosphor imager and a high resolution micro imager. Due to the very low activity of the samples, the sections on microscope slides were placed on a tritium-sensitive screen and stored for 14 days in a room with extremely low background radiation. The highest radioactivity was found in the intestinal region of the zebrafish (in the third, fourth and fifth section, which include the stomach). Bioconcentration of ivermectin seems to differ slightly between the three individual fish.

Figure 12. Results of the analyses of three ivermectin-exposed zebrafish with the phosphor-imager. The first number on each microscopic slide represents the number of the fish (1–3), the second number the serial number of the microscope slide containing the thin sections (1 is next to the head, and 6 or 8 next to the tail of the fish). Dark areas are caused by tritium-related radioactivity (from Wagner 2016).

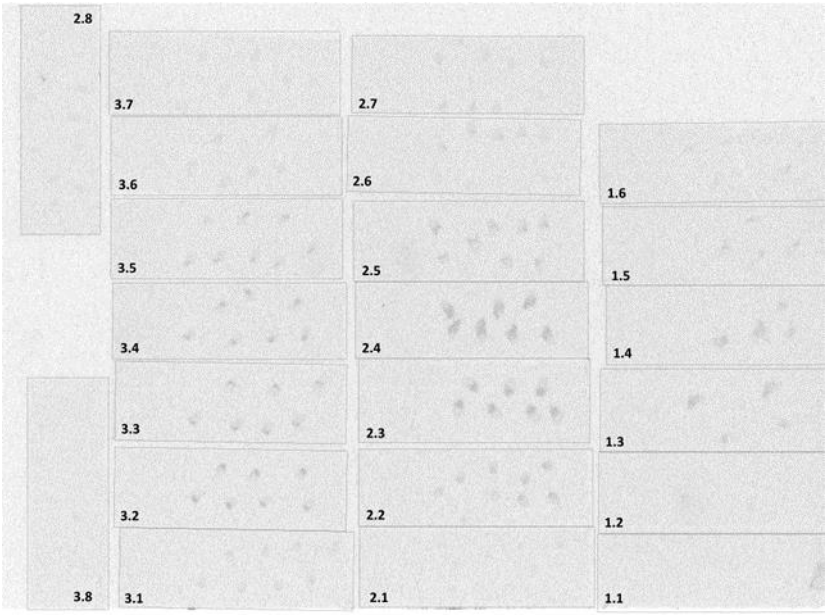

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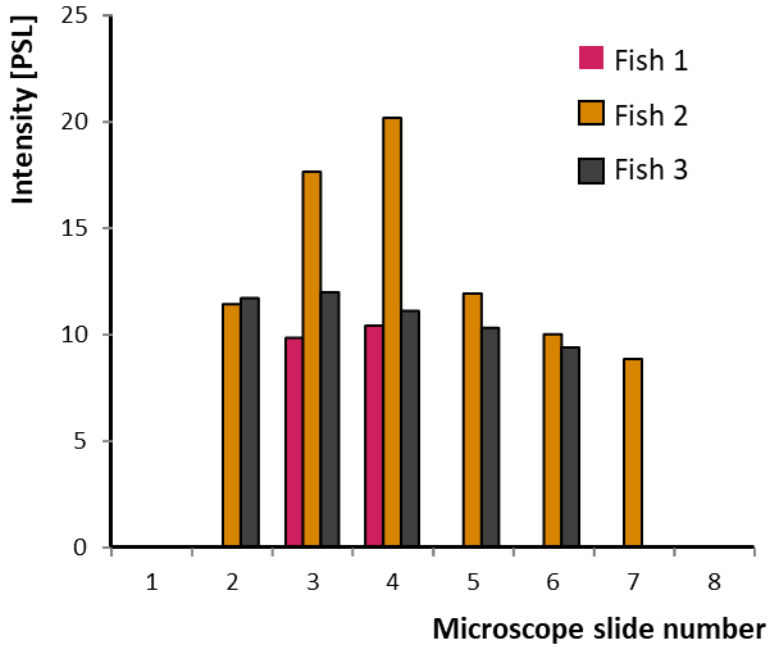
Figure 13. Radiation intensities (photostimulated luminescence, PSL) for each microscope slide (1 near the head, and 8 near the tail of the zebrafish).



Presentation from Wagner (2016).

A detailed description of the bioconcentration test is provided in the study report submitted to the UBA (Gilberg et al. 2017a). For a discussion of the test results, please see section 4.5.

4.4 Bioconcentration of ^{3}H-doramectin in zebrafish

The BCF of doramectin was determined according to OECD test guideline 305 (OECD 2012). A pre-test was performed with two doramectin concentrations (0.0081 and 0.081 µg/L). Due to the results of this pre-test, the definitive test was conducted at one concentration (0.041 µg/L, corresponding to 1000 dpm/mL), a water-only control and a solvent control. The uptake phase was terminated after 24 d of exposure; the subsequent depuration phase lasted 11 d. Fish and water samples were taken as described in Table 6.

Table 6: Sampling schedule for the definitive test with doramectin

	Doramectin (0.041 µg/L)
Uptake phase	
TRR Fish	d 1, 3, 7, 12, 14 and 20 (4 fish each), d 24 (6 fish)
TRR Water	daily (at least 2 samples)
PRP Fish	d 24 (28 fish)
PRP Water	d 24 (4 samples)
Depuration phase	
TRR Fish	d 0.17, 2, 4 and 8 (4 fish each), d 11 (6 fish)
TRR Water	d 0–5, 8 and 11 (2 samples each)

Abbreviations: d = day after start of exposure (uptake phase) and start of depuration phase, PRP = percentage of radioactivity associated with the parent compound, TRR = total radioactive residues

In the fish exposed to doramectin, no behavioural differences from the control fish were recorded. In all treatments and controls, no mortality was observed.

The doramectin concentrations measured in water were in the range of ± 20% of the mean measured concentration (1215 dpm/mL) on 20 of 23 sampling dates (see Figure 14). In individual samples, the measured concentrations were outside of this range (between 56 and 190% of the mean value). On days 8 and 9, the application solution was 4-fold overdosed due to a human failure. This led to increased concentrations in water on days 8–10 (Figure 14), and, subsequently, in fish on days 12 and 14. These values were excluded from estimation of kinetic parameters.

The verification of the steady state was made based on fish sampled on days 20 and 24 as well as on day 7 (the last sampling day before the overdosage), which had similar accumulation factors (see Figures 15 and 16).

Figure 14: Measured doramectin concentrations in the water during the definitive test with a doramectin concentration of 0.041 µg/L based on measured radioactivity (recovery indicated as % of mean measured radioactivity, 1215 dpm/mL; n = 2–10). The high concentration on days 8 and 9 are due to an overdosing of the application solution as discussed on the previous page.

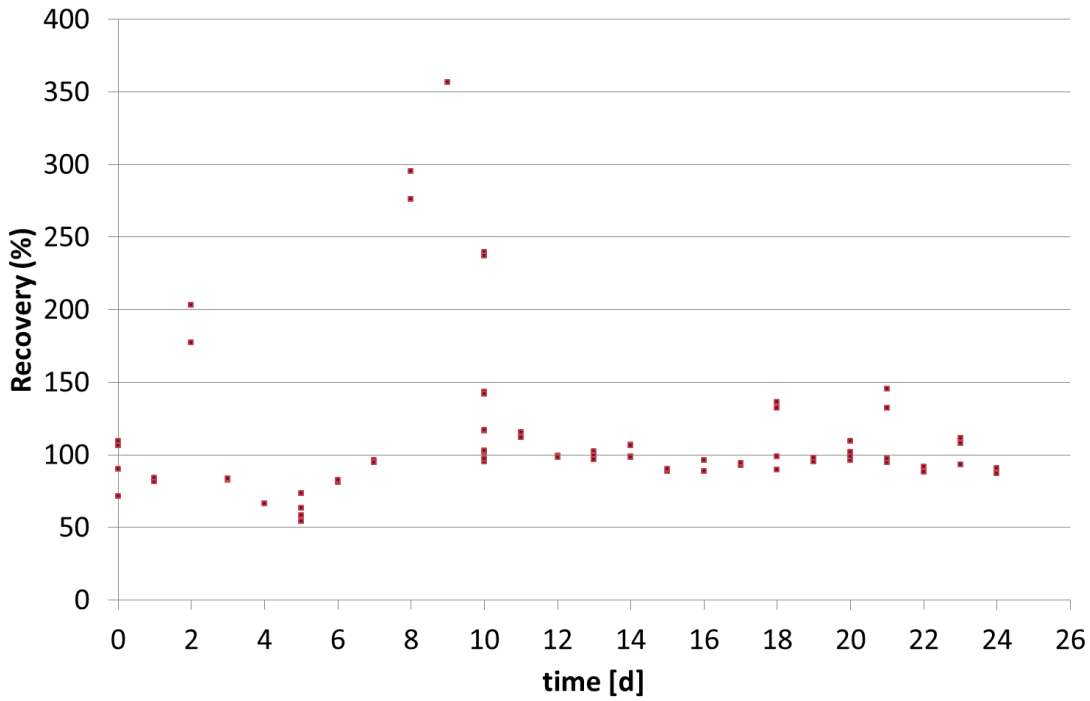


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Figure 15: Measured doramectin concentrations in the fish during the definitive test with a doramectin concentration of 0.041 µg/L (C_f in dpm/g fish wet weight). On day 7, one fish was identified as an outlier with about 750,000 dpm/g fish wet weight (n = 4 for days 1 to 20, n = 6 for day 24).

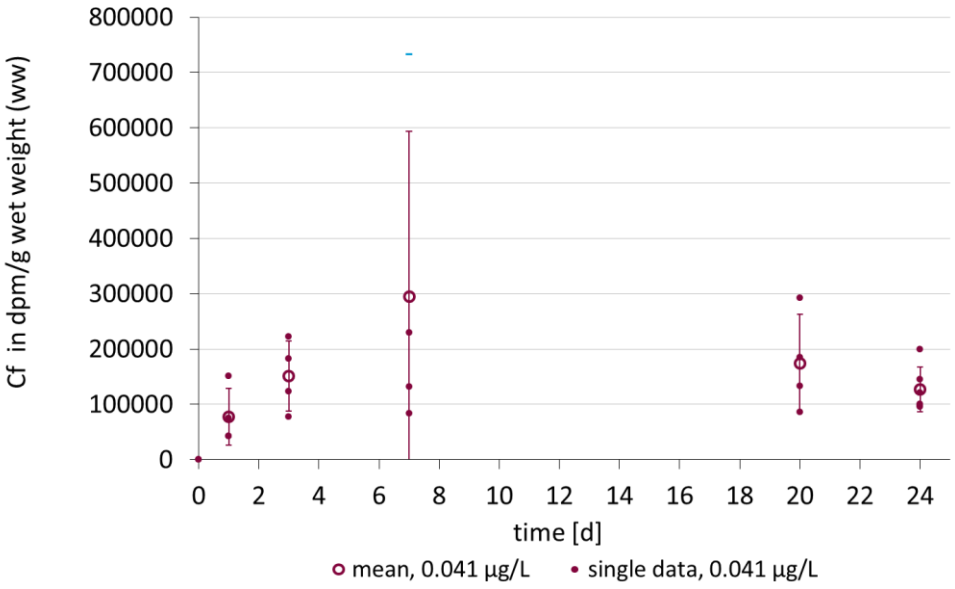

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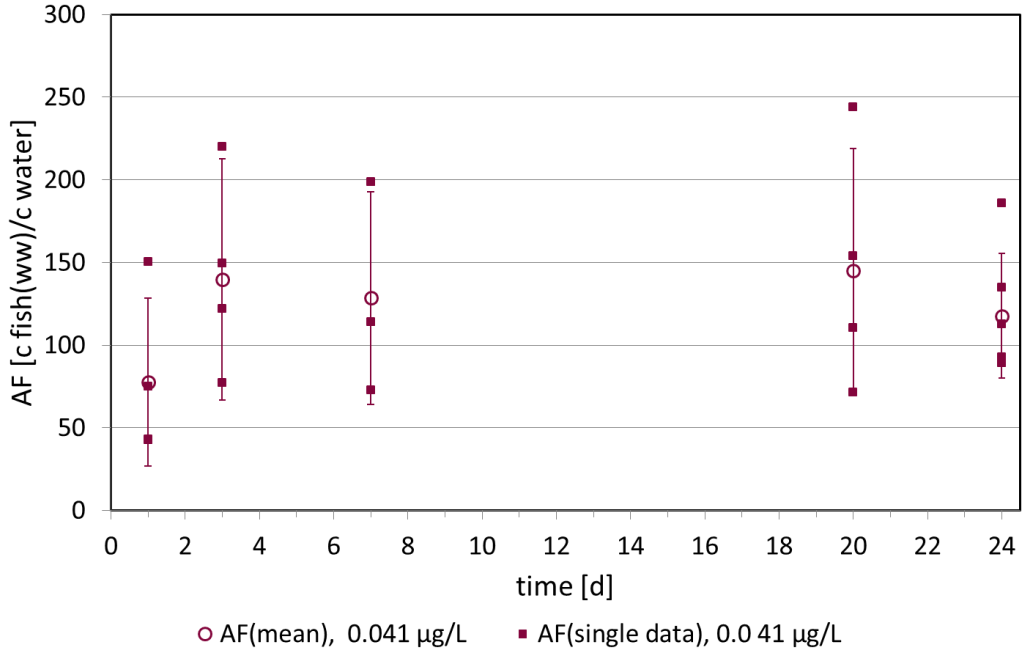
Figure 16: Individual and mean accumulation factors (AF = concentration in fish (C_f) / concentration in water (C_w)) with standard deviations in the definitive test at a doramectin concentration of 0.041 µg/L (n = 4, except for day 7 (n = 3)). Accumulation factors determined on days 12 and 14 (i.e. after the overdosage) were excluded.



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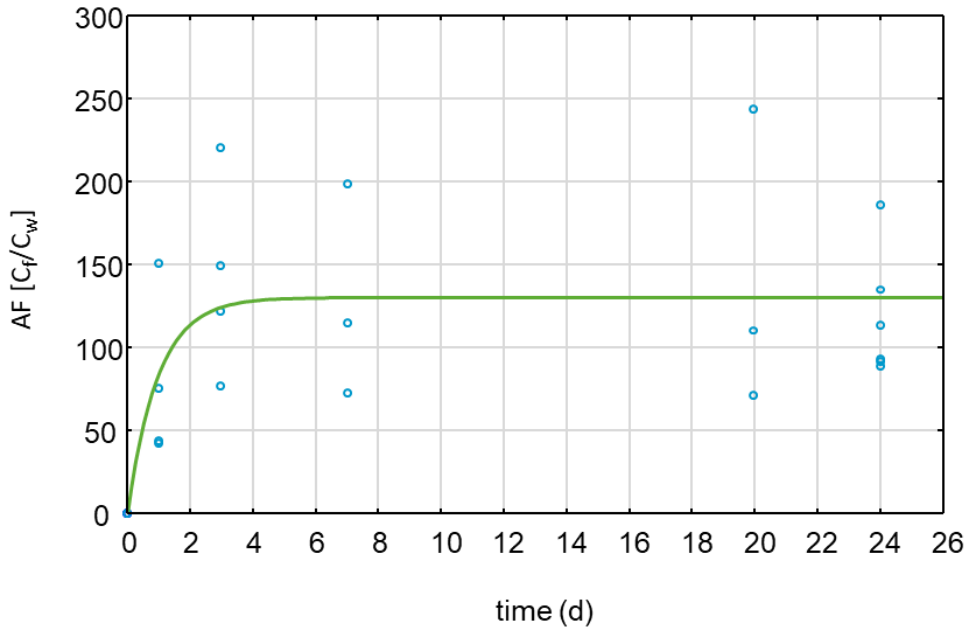
Figures 17 and 18 show the uptake and depuration kinetics based on accumulation factors.

Figure 17: Doramectin uptake kinetics at 0.041 µg/L (nonlinear regression analysis). The accumulation factors (AF) are based on total wet-weight-based concentrations in fish (C_f) and mean radioactive concentrations in water (C_w) at the corresponding sampling date.



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The kinetic bioconcentration factor (BCF_K) of 131 L/kg was calculated by linear regression analyses using the accumulation factors throughout the uptake phase. Due to varying doramectin concentrations in water, the calculation was based on the accumulation factors for individual fish (C_f / mean daily C_w). The determined BCF values (Table 7) are much lower than the threshold value for the B-criterion (BCF ≥ 2000 L/kg; EC 2011).

The time to reach 95% of steady state was 2.9 d as calculated according to OECD guideline 305 (OECD 2012).

Figure 18: Depuration kinetics for doramectin (0.041 µg/L) derived using nonlinear regression analysis based on radioactive concentrations (dpm/g) of doramectin in fish wet weight (C_f) throughout the depuration phase.

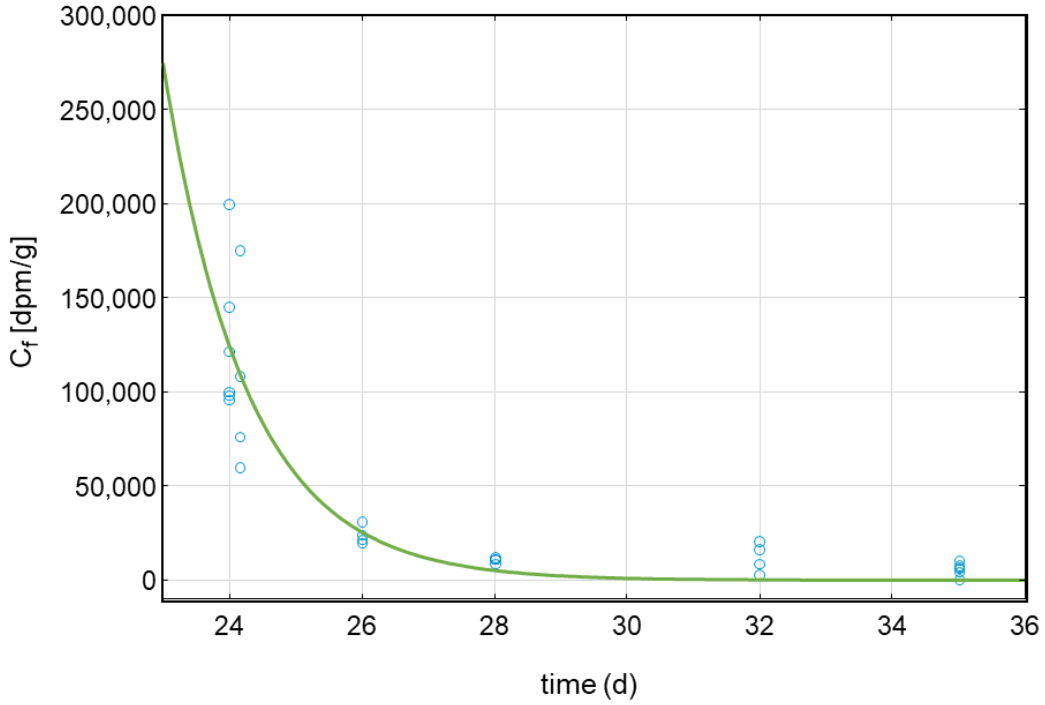

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Table 7: Summary of bioconcentration parameters for doramectin (nominal concentration in water: 0.041 µg/L) (uptake phase). All data are based on accumulation factors (wet-weight-based). All bioconcentration factors related to parent compound (BCF_{SSpc}, BCF_{Kpc}, BCF_{SSLpc}, BCF_{KLpc} and BCF_{KgLpc}) are based on the results of the differential chemical analysis that are described in the see next section (Table 8).

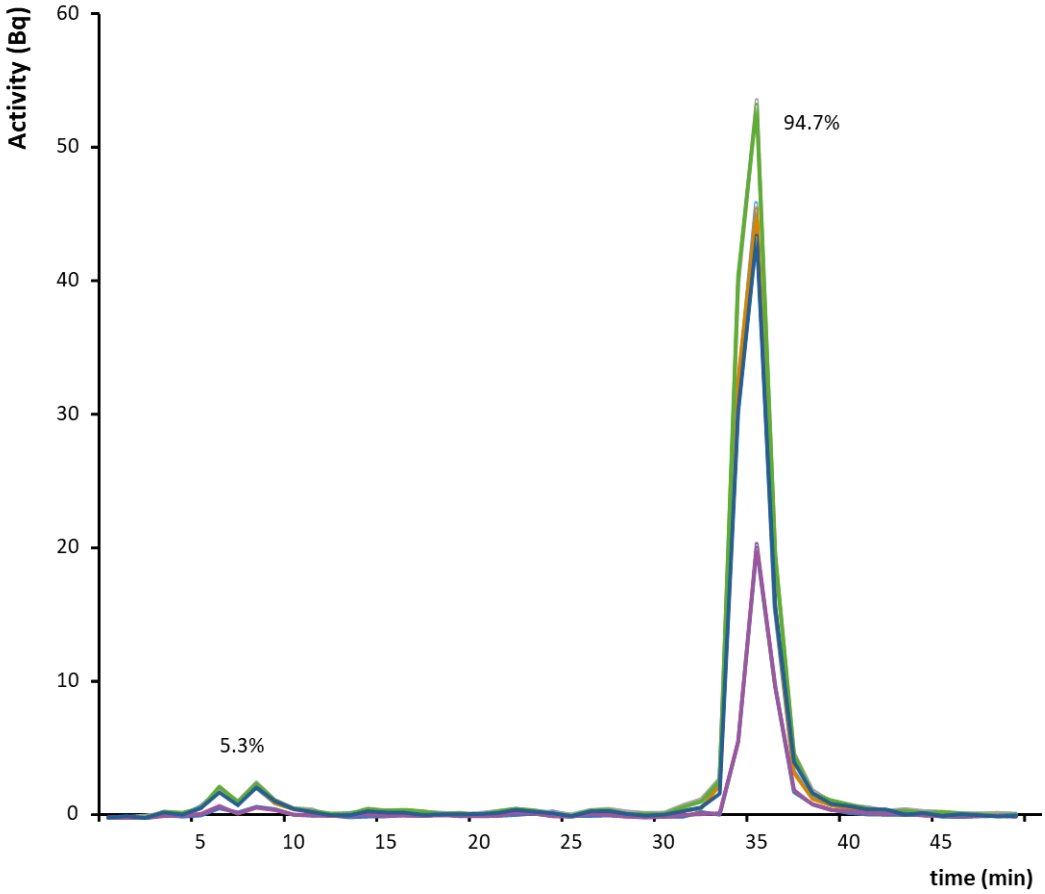
Parameters	Estimate	Lower asymptotic 95% confidence interval	Upper asymptotic 95% confidence interval	R / R^2
BCF_{SS}	129 ± 53 L/kg	-	-	-
BCF_K	131 L/kg	126	40	0.72 / 0.51
k_1	-135 L kg^{-1} d^{-1}	-275	4.8	
k_2	-1.03 d^{-1}	-2.19	0.118	
Time to reach 95% steady state	2.88 d	-	-	-
BCF_{SSL}	70 L/kg	-	-	-
BCF_{KL}	71 L/kg	-	-	-
BCF_{Kg}	131 L/kg	-	-	0.20 / 0.039
k_g	-0.00428 d_{-1}	-	-	
k_{2g}	-1.03 d^{-1}	-	-	
BCF_{KgL}	71 L/kg	-	-	-
BCF_{SSpc}	39.6 L/kg	-	-	-
BCF_{Kpc}	40.3 L/kg	-	-	-
BCF_{SSLpc}	21.6 L/kg	-	-	-
BCF_{KLpc}	40.5 L/kg	-	-	-
BCF_{KgLpc}	22.0 L/kg	-	-	-

Abbreviations: BCF_K = kinetic bioconcentration factor, BCF_{Kg} = growth-corrected kinetic bioconcentration factor, BCF_{KgL} = lipid-normalised growth-corrected kinetic bioconcentration factor, BCF_{KgLpc} = lipid-normalised and growth-corrected kinetic BCF of parent compound, BCF_{KL} = lipid-normalised kinetic bioconcentration factor, BCF_{KLpc} = lipid-normalised kinetic bioconcentration factor (BCF_K) of parent compound, BCF_{Kpc} = kinetic bioconcentration factor (BCF_K) of parent compound, BCF_{SS} = bioconcentration factor at steady state, BCF_{SSL} = lipid normalised bioconcentration factor at steady state, BCF_{SSLpc} = lipid-normalised steady-state bioconcentration factor (BCF_{ss}) of parent compound, BCF_{SSpc} = steady-state bioconcentration factor (BCF) of parent compound, k_1 = k_u = uptake rate constant, k_2 = k_e = depuration rate constant, k_{2g} = growth-corrected depuration rate constant, k_g = growth rate constant, R = coefficient of correlation, R^2 = coefficient of determination.

Results of the differential chemical analysis

At the end of the uptake phase, four water samples (500 mL) were taken and 120 mL isopropanol were added to each of the samples. The chromatograms shown in Figure 19 were obtained after solid phase extraction, HPLC and fractionated LSC. An average of 94.7% of the measured radioactivity was related to the main signal for doramectin at minute 36, while an average of 5.3% was related to two non-identified signals near the dead time (minute 8).

Figure 19: Combined radio-chromatograms of the water extracts from the definitive bioconcentration test with doramectin



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Four fish samples taken at the end of the uptake phase were extracted with acetonitrile and isopropanol. The chromatograms shown in Figure 20 were obtained after evaporation of the extraction solvent followed by HPLC and fractionated LSC. An average of 29.1% of the measured radioactivity was related to the main signal for doramectin (minute 36). An average of 60.9% of the radioactivity was related to non-identified signals near the dead time (minute 8). Three reproducible but also non-identified signals were found at minutes 17 (2.4%), 22 (2.8%) and 31 (2.9%).

Figure 20: Combined radio-chromatograms of the fish extracts from the definitive bioconcentration test with doramectin

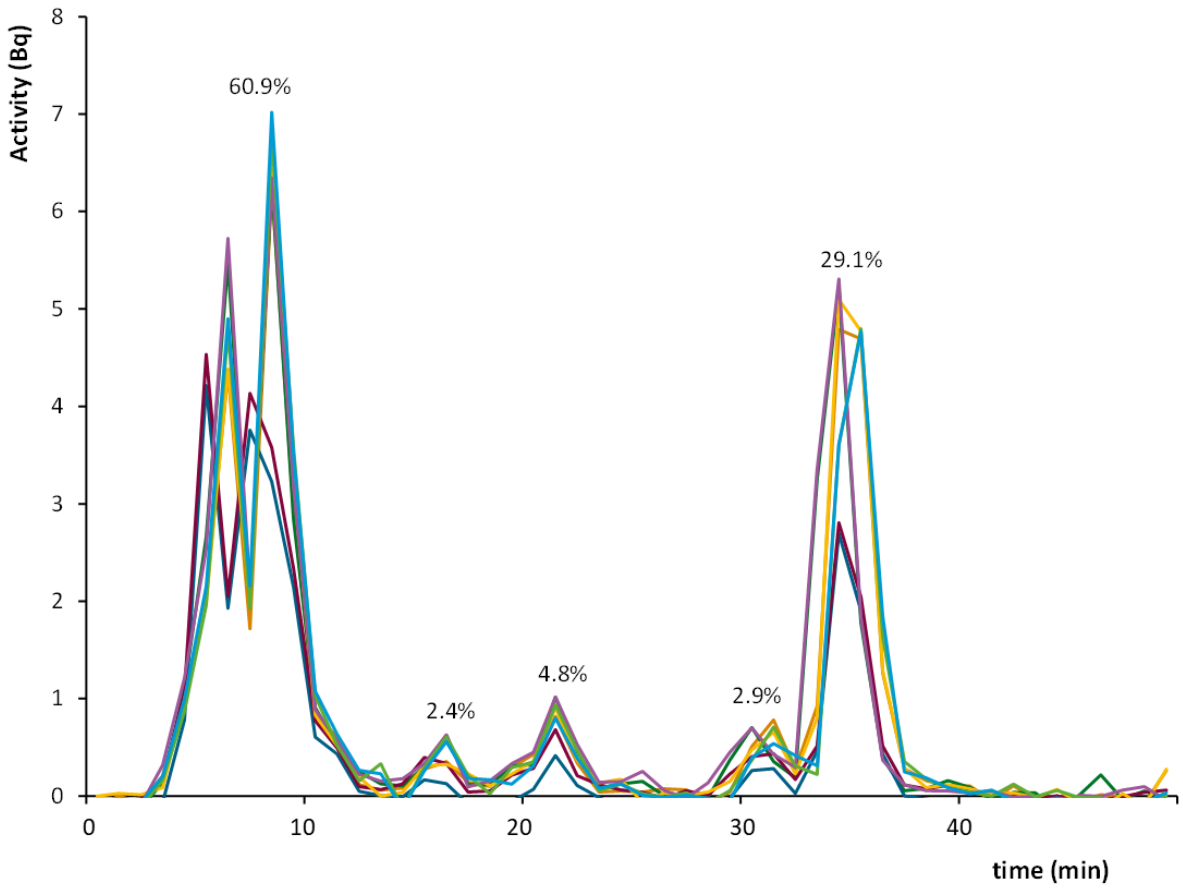

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Table 8: Overview of the mean percentages of parent compound and metabolites detected with specific chemical analysis in the definitive bioconcentration test with doramectin

	Parent compound	Further signals (metabolites)
Water samples	94.7%	5.3%
Fish samples	29.1%	70.9% (60.9 +- 2.4 + 4.8 + 2.9%)

A detailed description of the bioconcentration test is provided in the study report submitted to the UBA (Gilberg et al. 2017b); for a discussion of the test results, see section 4.5.

4.5 Discussion of the results of the bioconcentration studies with ivermectin and doramectin

For ivermectin, lipid-normalised bioconcentration factors of 63 (BCF_{SSL}) and 99 (BCF_{KL}) related to total radioactive residues were determined in zebrafish exposed to 0.01 µg/L (Table 3). The corresponding BCF values at the higher ivermectin concentration, 0.10 µg/L, were 82 (BCF_{SSL}) and 111 (BCF_{KL}; see Table 4). For doramectin, the respective BCF values are 70 (BCF_{SSL}) and 71 (BCF_{KL}; Table 7). These BCF values are much lower than expected based on an initial worst-case estimate derived using the equation indicated in EMEA/CVMP (2008) and the log P_{OW} values of 5.6 for ivermectin (section 3) and 4.4 for doramectin (US FDA 2002) [1] (with regard to these estimations, please also see section 3).

The bioconcentration factors derived in the present study for ivermectin and doramectin are in the same range as literature data for the related substance avermectin B_1. In bluegill sunfish (*Lepomis macrochirus*), BCF values of 52 (Wislocki et al. 1989) and 56 [2] (Van den Heuvel et al. 1996) for whole fish were determined in a flow-through test using ^{3}H-labelled avermectin B_{1a}. Both values relate to total radioactive residues. Growth rate and percentage of lipid were not determined (see Annex 1, Table 34). In sturgeon (the species is not indicated) exposed to avermectin B_1 at concentrations of 0.2 and 1.0 µg/L, similar BCF values of 42 and 41, respectively, were derived for muscle (Shen et al. 2005; cf. Annex 1, Table 34). As mentioned in section 4.1, no data on bioconcentration of ivermectin and doramectin in fish are publicly available.

For large molecules, steric hindrance of diffusion through membranes may lead to a reduced bioconcentration (Opperhuizen et al. 1985). Van den Heuvel et al. (1996) and Shen et al. (2005) hypothesized that in view of the molecular dimensions of 1.7 x 1.9 x 1.8 nm such a steric hindrance might have been the cause of the low BCF values determined for avermectin B_1. De Wolf et al. (2007) stated that a molecular length of ≥ 4.3 nm could be used as indicator that a substance is unlikely to bioconcentrate, while a diameter of ≥ 1.74 nm combined with a molecular weight of 700–1000 g/mol might indicate a BCF < 2000. However, Arnot et al. (2010) highlighted that there is evidence that substances being larger than the suggested cut-off values are adsorbed and accumulated.

As mentioned in section 4.1, a BCF > 2000 has been determined for the milbemycin moxidectin (EMA/CVMP 2016a). This value is much higher than the bioconcentration factors for the three avermectins (ivermectin, doramectin and avermectin B_1), which are around or below 100 (see Table 9). Moxidectin has a somewhat lower molecular size and weight (640 g/mol) than ivermectin (875 g/mol), doramectin (899 g/mol) and avermectin B_1 (873 g/mol). The most conspicuous difference in molecular structure between the avermectins and moxidectin is the lack of the disaccharide moiety in the latter (see Table 9). In their review on moxidectin and avermectins, Prichard et al. (2012) pointed out that this disaccharide moiety is the reason for a different affinity of the avermectins and moxidectin to P-glycoprotein [3] (also referred to as multidrug resistance protein 1). P-Glycoprotein is an ATP-dependent transmembrane transporter, which is especially found in membranes of organs with adsorption, elimination or barrier functions. Studies on mammals have shown that P-glycoprotein is an effective efflux pump for ivermectin (Boelsterli 2005, Prichard et al. 2012). Due to the absence of the disaccharide moiety, moxidectin interacts more weakly with P-glycoprotein than ivermectin (Table 9). Therefore, it is eliminated to a much lower extent (Prichard et al. 2012).

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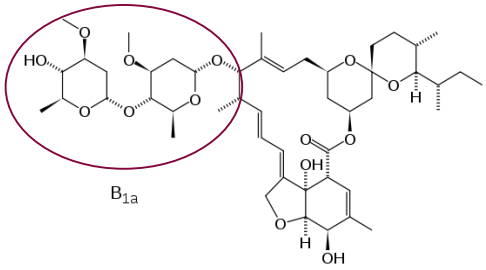
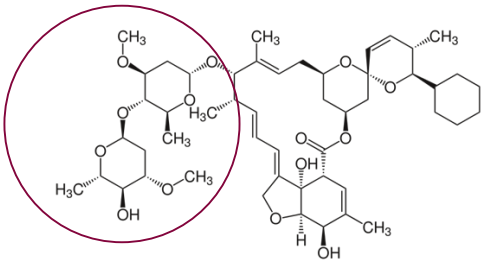
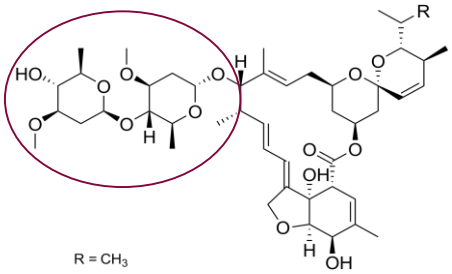
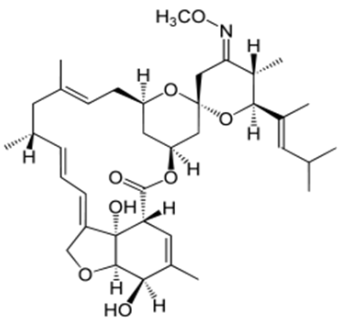
[1] According to EMEA/CVMP (2008), the equation developed by Veith et al. 1979 ($\log BCF_{fish} = 0.85 \cdot \log K_{OW} - 0.70$) can be used to estimate the BCF for substances with log P_{OW} values between 2 and 6 and a molecular weight <700 g/mol. For substances with a higher molecular weight such as ivermectin and doramectin, this equation can be used as initial worst-case estimate. Using this equation and the log P_{OW} values indicated above, BCF values of approx. 11,000 and 1,000 are derived for ivermectin and doramectin, respectively.

[2] Both BCF values were derived from the same study (see Annex 1, Table 34).

[3] Please note that P-glycoprotein also occurs in invertebrates and that it is relevant for some resistances of parasites against macrocyclic lactones (see section 6).

Table 9. Overview of bioconcentration factors in fish, molecular weight, molecular structure and interaction with P-glycoprotein for ivermectin, doramectin, avermectin B_1 and moxidectin. While the disaccharide moiety is present in the three avermectins (red circles), it is lacking in moxidectin.

Substance	BCF in fish (L/kg)	Molecular weight	Molecular structure	Interaction with P-glycoprotein
Ivermectin	63-111 [a]	875		Strong [d]
Doramectin	70-71 [a]	899		Strong [d]
Avermectin B_1	41-56 [b]	873		Strong [d]
Moxidectin	> 2000 [c]	640		Weak [d]

[a] Lipid-normalised bioconcentration factors related to total radioactive residues determined in the present study.

[b] Wislocki et al. 1989, Van den Heuvel et al. 1996, Shen et al. 2005

[c] EMA/CVMP 2016a

[d] Based on Prichard et al. 2012

Given that fish also possess P-glycoprotein (Sturm & Segner 2006), it can be assumed that this transporter is effectively eliminating ivermectin and the structurally related doramectin from exposed fish and that this active efflux is the main reason for the low BCF values. This hypothesis is supported by the rapid depuration of both ivermectin and doramectin by zebrafish that was demonstrated in the present study (Figures 8, 9 and 18).

The much higher BCF of 750 determined by Davies et al. (1997) in blue mussels (*Mytilus edulis*; see section 4.1 and Annex 1, Table 34) might be related to the fact that molluscs have limited capacities to metabolise and excrete organic chemicals (see e.g. Lee 1986, Oehlmann et al. 2007).

In the present study, thin sections of exposed zebrafish were analysed using a phosphor imager and a high resolution micro imager to evaluate spatial distribution of ivermectin in the fish. Most ivermectin was accumulated in the intestinal region of zebrafish (see section 4.3, Figures 12 and 13). This finding is in line with the results of Van den Heuvel et al. (1996), who determined BCF values of 84 in viscera and 28 in muscle. However, in this context it should be mentioned that high ivermectin concentrations were also found in the central nervous system of fish following administration by stomach tubing and intraperitoneal injection. This finding indicates that ivermectin distribution is not limited to the gut region, but that it can be transported to various body compartments (Høy et al. 1992, Katharios et al. 2004).

5 Overview of the use of macrocyclic lactones for the treatment of pasture animals

Information on the clinically most important parasites in horses, cattle, and sheep and on the parasiticides used for their control was compiled by the University of Hohenheim. Within these parasites, the helminths predominate. Most of them are distributed worldwide (e.g. Ascaridae and Strongolidae) with prevalence rates varying widely in global terms (e.g. prevalences between 22 to 80% for *Parascaris equorum*). The reason for this lies in different land uses and agricultural structures (e.g. pasture vs. stable). In addition, pastures with marshy meadow soil and pastures around creeks, rivers or drainages are a main predisposing factor for parasitoses of grazing animals. Table 10 provides an overview of the most important parasites in pasture animals, their prevalences worldwide and the used parasiticides.

Table 10: Overview of the most important parasites in horses, cattle, and sheep and the parasiticides used for their control (according to Deplazes et al. 2013)

Parasite	Occurrence and prevalence in Europe	Used parasiticide (anthelmintic)
Horses		
Ascaridae *Parascaris equorum*	Occurrence: worldwide Prevalence: 22–80%, young animals with higher prevalences	Benzimidazoles, macrocyclic lactones, pyrimidines
Strongylidae Large strongylae: *Strongylus vulgaris*, *Strongylus edentatus*, *Strongylus equinus* Cyathostominae (small strongylae): at least 51 species	Occurrence: worldwide. Variable prevalences from < 10% to nearly 100% depending on deworming regime and management Occurrence: worldwide. High prevalences (up to 100%)	Benzimidazoles, macrocyclic lactones, pyrimidines
Cestoda *Anoplocephala perfoliata* *Anoplocephala magna* *Paranoplocephala mamillana*	Variable prevalences (up to 70%)	Pyrimidines, praziquantel
Cattle, sheep		
Coccidia *Eimeria* spp.	Occurrence: worldwide. Prevalences in Germany in conventional husbandries: 3–25%, in suckler cow husbandries and organic beef production up to 90%	Toltrazuril, sulphonamides
Fasciola hepatica	Occurrence: worldwide Prevalence in Germany: 1–17%	Triclabendazol, closantel
Dicrocoelium dendriticum	Occurrence: in Europe, Asia, North Africa, North America Prevalence in Germany: 0–25%	Benzimidazoles (high dosage)

Parasite	Occurrence and prevalence in Europe	Used parasiticide (anthelmintic)
Paramphistomum spp.	Occurrence: worldwide. Prevalence in North Germany: 1–60%	Benzimidazoles (high dosage)
Dictyocaulus viviparus (cattle) *Dictyocaulus filaria* (sheep)	Occurrence: worldwide. Prevalence: 15–70%	Benzimidazoles, macrocyclic lactones, imidazothiazoles (e.g. levamisole)
Moniezia spp.	Prevalence in Germany: about 10%	Benzimidazoles
Trichostrongylidae *Haemonchus, Ostertagia* *Cooperia, Nematodirus*	Prevalence highly variable	Benzimidazoles, macrocyclic lactones, imidazothiazoles (e.g. levamisole), tetrahydropyrimidine

Macrocyclic lactones (avermectins and milbemycins) are used especially for the treatment of parasitoses caused by Ascaridae and Strongylidae in horses and by Trichostrongylidae (*Haemonchus*, *Ostertagia*, *Cooperia*, *Nematodirus*) in cattle and sheep (Tables 10 – 12). Based on the respective animal species (cattle, sheep, or horse) and indication (parasite species) in combination with type of livestock and type of management, the most effective parasiticide and best available formulation/application form (dermal or parenteral application) should be chosen. Both, avermectins and milbemycins, are used worldwide to treat cattle, sheep, and horses against ecto- and endoparasites using dermal or parenteral application forms. Detailed information on the possible use of ivermectin, doramectin, eprinomectin and moxidectin including the animal species, the indication, dosage, and treatment strategy is provided in Tables 11 and 12.

Table 11: Detailed information of the application of avermectins in horses, cattle, and sheep worldwide. Please note that the parasiticides included in this table are not necessarily approved for treating pasture animals in Germany

Avermectin	Animal species	Indication	Route of application	Dosage, contraindication	Treatment	Single animal or herd treatment
Doramectin	Sheep	**Ectoparasites** *Psoroptes ovis*[1]	i.m.	Dosage: 0.3 mg/kg bw Withdrawal period: meat and offal 42 d Contraindication: lactating animals; dry dairy ewes within 60 d before lambing	At time of diagnosis one application	Herd
	Goat	**Ectoparasites** botflies (*Przhevalskiana silenus*)[2]	s.c.	Dosage: 0.2 mg/kg bw Contraindication: lactating animals; dry dairy goats within 60 d before lambing	At time of diagnosis one application	Herd
	Cattle	**Gastrointestinal tract** nematodes (adults and immature stages; *Ostertagia* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Cooperia* spp., *Bunostomum phlebotomum, Oesophagostomum radiatum, Nematodirus* spp., *Strongyloides papillosus, Trichuris* spp.)[3] **Respiratory tract** *Dictyocaulus viviparus*[4] **Eyes**[5] *Thelazia* spp.	Dermal (pour-on); s.c.	Dermal: 0.5 mg/kg bw; s.c.: 0.2 mg/kg bw Withdrawal period: meat and offal 49 d. Contraindication: lactating animals; dry dairy cows including pregnant heifers within 60 d before calving	Protection against for *Ostertagia ostertagi* 35 d *Cooperia oncophora* 28 d *Dictyocaulus viviparus* 42 d *Haematobia irritans* 42 d *Damalinia bovis* 42 d *Trichostrongylus axei* 28 d *Linognathus vituli* 49 d *Solenopotes capillatus* 35 d	Herd

Avermectin	Animal species	Indication	Route of application	Dosage, contraindication	Treatment	Single animal or herd treatment
		Ectoparasites [6] mites (*Sarcoptes scabiei, Psoroptes bovis, Chorioptes bovis*) lice (*Haematopinus eurysternus, Linognathus vituli, Solenopotes capillatus*) ticks (*Boophilus microplus*) flies (*Haematobia irritans*)	s.c.	Dosage: 0.2 mg/kg bw Contraindication: lactating animals (last 60 d of pregnancy)	n.a.	
Eprinomectin	Cattle	**Gastrointestinal tract**[7] larvae and adults of *Haemonchus* spp., *Ostertagia* spp., *Cooperia spp., Trichostrongylus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Bunostomum* spp., *Trichuris* spp. **Respiratory tract**[8] *Dictyocaulus viviparus* **Skin and connective tissue**[9] *Hypoderma* spp. **Ectoparasites**[10] lice and biting lice (*Linognathus vituli*, *Haematopinus eurysternus, Solenopotes capillatus, Damalinia bovis*)[13] mites (*Sarcoptes bovis, Chorioptes bovis*)[14] flies (*Haematobia irritans*)[15]	Dermal (pour-on)	Dosage: 0.5 mg/kg bw Contraindication: lactating animals (last 60 d of pregnancy)	**Nematodes**: first day of pasture, and 2 or 8 weeks later[19] **Ectoparasites**: one application is effective for approx. 8 weeks[20]	Herd

Avermectin	Animal species	Indication	Route of application	Dosage, contraindication	Treatment	Single animal or herd treatment
Ivermectin	Cattle	**Nematodes**[11] (*Haemonchus* spp., *Ostertagia* spp., *Trichostrongylus* spp., *Cooperia* spp., *Oesophagostomum* spp., *Nematodirus* spp., *Bunostomum* spp., *Toxocara* spp., *Trichuris* spp.) **Respiratory tract**[12] *Dictyocaulus viviparus*[17] **Ectoparasites**[13] mites (*Psoroptes* spp./ *Sarcoptes* spp.) *Chorioptes bovis* lice (*Haematopinus* spp., *Linognathus* spp., *Solenopotes capillatus*) flies	s.c. oral (gel)	Dosage: 0.2 mg/kg bw Withdrawal period: meat and offal 49 d Contraindication: lactating dairy cows; non-lacatating dairy cows (including pregnant dairy heifers) within 60 d of calving	Single application	Herd
	Sheep	**Gastrointestinal tract** *Ostertagia circumcinta, Haemonchus contortus, Trichoytrongylus axei, T. colubriformis, T. vitrinus, Cooperia curticei, Nematodirus filicollis* **Respiratory tract** *Dictyocaulus filarial* **Ectoparasites** mites (*Psoroptes ovis*) nasal bot fly (*Oestrus ovis*)	s.c.	Dosage: 0.2 mg/kg bw Withdrawal period: meat and offal 42 d Contraindication: lactating dairy ewes; dry dairy ewes within 60 d of lambing	Single application, except for treatment of *Psoroptes ovis* (sheep scab): two injections with a 7-d interval are required to treat clinical signs of scab and to eliminate living mites	Single and herd

Avermectin	Animal species	Indication	Route of application	Dosage, contraindication	Treatment	Single animal or herd treatment
Ivermectin (continued)	Horses	**Gastrointestinal tract**[16] *Strongylus* spp., *Oxyuris equi, Parascaris equorum, Strongyloides* spp., *Trichostrongylus* spp., *Gasterophilus*-larvae **Respiratory tract**[17] *Dictyocaulus arnfieldi, Gasterophilus nasalis* larvae **Ectoparasites**[18] mites (*Sarcoptes, Proroptes*) lice	oral (paste) oral (tablets)	Dosage: 0.2 mg/kg bw (for both paste and tablets)	Faeces remain 8-9 weeks negative for nematode eggs[21]. The therapy is repeated after 12-14 weeks[22].	Single and herd (depending on the age, diagnostic results, and season)

Abbreviations: bw = body weight, i.m. = intramuscular injection, s.c. = subcutaneous injection.

1 Bates et al. 1995

2 Niutta et al. 1997

3 Mehlhorn et al. 1993, Yazwinski et al. 1994a, Jones et al. 1993, Eddi et al. 1993, Goudie et al. 1993, Yazwinski et al.1994b, Eddi et al. 1997; Yazwinski et al.1997a

4 Weatherley et al. 1993, Eddi et al. 1993, Barton et al. 1995, Goudie et al. 1993, Yazwinski et al. 1997b

5 Kennedy & Philipps 1993

6 Logan et al. 1993, Scheffler 1995, Losson et al. 1998, Villeneuve & Daigneault 1997, Gonzalez et al. 1993, Muniz et al. 1995

7 Pitt et al. 1997; Williams et al. 1997a; Yazwinski et al. 1997a, Gogolewski et al. 1997, Shoop et al. 1996, Epe et al. 1999

8 Yazwinski et al. 1997b, Shoop et al. 1996, Pitt et al. 1997, Epe et al. 1999

9 Holste et al. 1998

10 Holste et al. 1997, Shoop et al. 1996, Barth et al. 1997

11 Egerton 1981, Alva-Valdes et al. 1986, Prichard 1988, Williams et al. 1981, Armour & Bairden 1980, Williams & Plue 1992, Williams et al. 1997b, Van Miert et al. 1994, Yazwinski et al.1997b

12 Lyons et al. 1981, Sutherland 1990, Alva-Valdes et al. 1986, Borgsteede 1993, Rehbein et al. 1997, Williams et al. 1997b

13 Guillot & Meleney 1982, Meleney 1982, Sutherland 1990, Wright & Guillot 1984b, Rehbein et al. 1997, Lonneux et al. 1997, Barth & Preston 1988, Titchener 1985, Meyer 1980

14 Todd et al. 1985, Swan et al. 1984, Armour & Bairden 1982, Sutherland & Campbell 1990, Borgsteede 1993, Bogan et al. 1988

15 Swan et al. 1984, Sutherland 1990, McCraw & Menzies1986, Van Miert & van Meer 1994

16 DiPietro et al. 1982, Sutherland 1990, Lyons et al. 1992, Craig & Kunde 1981, Klei 1980, Lyons et al. 1980, Torbert et al. 1982, Mogg 1990, Xiao 1994, Lo et al. 1985, Hasslinger 1982, Lyons et al. 1992, Rolfe 1998, Lyons et al. 1982, Demeulenaere et al. 1997, Van Doorn et al. 2012, Austin et al. 1991, Egerton 1981, Lyons et al. 1980, Ungemach 1994

17 Britt & Preston 1985, Sutherland 1990, Bello 1981, Craig & Kunde 1981, Egerton 1981, Lyons et al. 1980, Torbert et al. 1982, Sutherland 1990

18 Plumb 1991, Ungemach 1994, Eckert et al. 1999

[19] Epe et al. 1999

[20] Holste et al. 1997, Shoop et al. 1996, Barth et al. 1997

[21] Boersema et al. 1998, Piche et al. 1991, Lumsden et al. 1989, Parry et al. 1993, Mogg 1990, Austin et al. 1991, Boersema et al. 1996, Rolfe 1998

[22] Jacobs et al. 1995

Table 12: Detailed information of the application of moxidectin in horses, cattle, and sheep worldwide. Please note that the parasiticides included in this table are not necessarily approved for treating pasture animals in Germany

Milbemycin	Animal species	Indication	Route of application	Dosage, contraindication	Treatment	Single animal or herd treatment
Moxidectin	Cattle	**Gastrointestinal tract**[1] *Ostertagia ostertagi, Haemonchus* spp., *Cooperia* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Bunostomum* spp., *Capillaria* spp., *Trichuris* spp. **Respiratory tract**[2] *Dictyocaulus viviparus* **Skin and connective tissue**[3] *Hypoderma* spp. **Ectoparasites**[4] mites (*Chorioptes bovis, Psoroptes ovis, Sarcoptes scabiei*) lice (*Linognathus vituli*) ticks (*Boophilus microplus)*	s.c. Dermal (pour-on)	Dosage: 0.2 mg/kg bw Dosage: 0.5 mg/kg bw	Ectoparasites: residual effect for at least 8 weeks[10]	Herd
	Horses	**Gastrointestinal tract**[5] *Strongylus* spp., *Parascaris equorum*[2] *Oxyuris equi* *Trichostrongylus axei* *Gasterophilus* spp. *Habronema muscae* **Respiratory tract** Dictyocaulus spp.[6]	Oral (gel)	Dosage: 0.4 mg/kg bw	Preventive application: 0.4 mg/kg bw every 4 weeks[11]	Single/herd
	Sheep	**Gastrointestinal tract**[7] *Haemonchus contortus, Ostertagia* spp., *Trichostrongylus* spp., *Cooperia* spp., *Teladorsagia* spp., *Trichuris* spp. **Respiratory tract**[8] lungworms (*Cystocaulus ocreatus, Muellerius capillaris, Neostrongylus linearis, Protostrongylus rufescens*)[15] **Ectoparasites**[9] mites (*Psoroptes ovis, Sarcoptes scabiei*)[16]	Oral (solution)	Dosage: 0.2 mg/kg bw		

Abbreviation: bw = body weight

[1] Eysker et al. 1996, Zimmermann et al. 1992, Hubert et al. 1997, Williams et al. 1996, Morin et al. 1996, Whang et al. 1994, Williams and Plue 1992, Ranjan et al. 1992, Eysker & Eilers 1995, Hubert et al. 1995a

[2] Hubert et al. 1997, Williams et al. 1996, Williams & Plue 1992, Eysker & Eilers 1995, Hubert et al. 1995b

[3] Boulard et al. 1998, Lonneux & Losson 1994, Scholl et al. 1992

[4] Losson & Lonneux 1996, Losson & Lonneux 1993, Lonneux & Losson 1992, Scheffler 1995, Lonneux et al. 1997, Titchener 1994, Guglielmone et al. 2000

[5] Dorchies et al. 1998, Eysker et al. 1997, Monahan et al. 1996, Monahan et al. 1995a, Lyons et al. 1992, Bauer & Conraths 1998b, Xiao 1994, Coles et al. 1998, DiPietro et al. 1997, Boersema et al. 1998, Monahan et al. 1995b, Bauer & Conraths 1998, Grubbs et al. 2003, Xiao 1994, Hubert et al. 1997, Scholl et al. 1998,

[6] Coles et al. 1998

[7] Uriarte et al. 1994, Kerboeuf et al. 1995a, Coles et al. 1994, Peter et al. 1994, Kerboeuf et al. 1995b, Bauer & Conraths 1994

[8] Papadopoulos et al. 2004

[9] Corba et al. 1995, O`Brien et al. 1994, O`Brien et al. 1996, Williams & Parker 1996, Fthenakis et al. 2000, Papadopoulos et al. 2000

[10] Losson & Lonneux 1993, 1996; Lonneux & Losson 1992; Polley et al. 1998, Lonneux et al. 1997

[11] Demeulenaere et al. 1997, Rolfe 1998

6 Anthelmintic resistances to macrocyclic lactones with a focus on avermectins and milbemycins used in pasture animals: overview and consequences for risk management strategies

Since resistances of the parasites to the active pharmaceutical agent are relevant when discussing possible risk management strategies, an overview of anthelmintic resistances to avermectins and milbemycins was prepared by the University of Hohenheim. Resistances against veterinary pharmaceuticals are found in protozoan and metazoan parasites. If a resistance occurs in a parasite, a (sub-) population of this parasite species is able to tolerate a dose of an antiparasitic that is normally lethal for most individuals of a susceptible population. Resistances are widely spread in parasite populations, and cross-resistances or multiple resistances are also found. Resistances against anthelmintics are described throughout Europe, mainly against benzimidazoles, but also against imidathiozoles and macrocyclic lactones. These resistances are detected, because deworming measures do not work anymore – often without laboratory evidence. In Central Europe, resistances can be found in *Trichostrongylus* species infesting horses, sheep and goats. For the latter, multiple resistances have often been found (e.g. in UK, France and Denmark; Peña-Espinoza et al. 2014, Learmount et al. 2016, Paraud et al. 2016).

Resistances to macrocyclic lactones

The occurrence of resistances against ivermectin and moxidectin and their spreading has been described over the last few years for small ruminants and cattle but also for horses. However, reliable data on the prevalence of resistances against macrocyclic lactones in parasites and on a possible reversibility of these resistances are not available.

In the UK and in Italy, ivermectin-resistant *Trichostrongylus* species can be found in sheep. In Spain, ivermectin resistances have been reported for *Teladorsagia* species, in Sweden for *Haemonchus* species. In Switzerland and southern Germany, these nematodes also exhibited resistances against moxidectin (Scheuerle et al. 2009). Resistances to doramectin are also found (e.g. in the Netherlands; Borgsteede et al. 2007). Recently, resistances against macrocyclic lactones, especially ivermectin, have increased. However, the main problem appears to be the accumulation of multi-resistant isolates (Ramünke et al. 2016).

Despite these resistances, the described antiparasitics are still in use. Although cross-resistances are found, moxidectin seems to be more effective against resistant parasitic isolates in sheep, goats, cattle and horses (see below).

Resistance development

As mentioned by Eckert et al. (2008) and Rose et al. (2015), the development of resistances to parasiticides is promoted by the following factors: a high frequency of treatment, long-term usage of the same active ingredients, under-dosing, short generation times of the parasites and insufficient refugia, in which susceptible parasites survive (e.g. untreated animals, larvae on grazing land).

The identification of the mechanisms causing resistances in parasites is still a challenge in parasitology. There is evidence that so-called ATP-binding cassette (ABC-) transporters such as P-glycoprotein, an ATP-dependent transmembrane transporter (Boelsterli 2005, Prichard et al. 2012; see also section 4.5), play a critical role in the development of resistances in several nematode species (Bygarski et al. 2014). These resistances often concern several parasiticides. The gene expression level for these transporters and their allele frequencies were modified in *Haemonchus contortus* and *Cooperia oncophora*, respectively, exhibiting a higher tolerance against macrocyclic lactones. In addition, a muta-

tion in the *dyf-7* gene [4], which leads to an abnormal morphology of the sensory neurons, was found in ivermectin-resistant *Caenorhabditis elegans* and *Haemonchus contortus*. The induction of the detoxification system and malfunction in the integrity of chemosensory neurons are two mechanisms that seem to influence the susceptibility of nematodes towards ivermectin and moxidectin. Modulations in the metabolism of xenobiotics were also found (Kotze et al. 2014).

In vitro studies with *C. elegans* clearly showed that both ivermectin and moxidectin can induce cross-resistances against macrocyclic lactones (Ménez et al. 2016). Despite such cross-resistances between ivermectin and moxidectin, moxidectin appears to be less affected by resistances than ivermectin. Within different *Strongylus* species, resistance towards ivermectin is more widespread than resistance towards moxidectin. Moxidectin is still more effective against resistant isolates in sheep, goats, cattle, horses and dogs. However, the molecular mechanisms that lead to this selection of resistances and the mechanisms of cross-selection have to be investigated further. The fact that ivermectin is strongly interacting with P-glycoprotein, while interactions of moxidectin with this transmembrane transporter are only weak (Prichard et al. 2012; see section 4.5) could contribute to the observed differences.

Diagnosis of parasitic resistance

The possibility to detect resistances in parasites is still very limited, since the currently used *in vivo* and *in vitro* methods are limited in their sensitivity, specificity and diagnostic value (i.e. the interpretation of the diagnostic result is not always straightforward).

In vitro methods comprise the so-called egg reduction test, the larval hatching test and the larval development test that are used to perform a diagnosis in a living population of host animals. In the egg reduction test, the egg count in faeces is determined before and after treatment with a parasiticide, and a reduction rate is calculated. In macrocyclic lactones, reduction rates below 95% indicate a drug resistance. The egg reduction test is only suitable for presumptive resistance diagnostics (De Graef et al. 2012). For exact examinations, it is necessary to perform more precise laboratory studies (i.e. the larval hatching or development test) or even animal experiments (*post mortem* diagnostics). For *post mortem* diagnostics, the animals have to be euthanised and the affected organs are prepared for counting the developmental stages of the parasites. Further establishing and routine use of molecular assays based on genetic resistance markers can be expected to improve diagnosis of parasitic resistance in the future (Demeler et al. 2013, Knapp-Lawitzke et al. 2015, Ramünke et al. 2016).

Measures against the development and spreading of parasitic resistances

Approaches to avoid resistances against parasiticides are most promising, if they aim at preventing a selective pressure on the parasites and at preserving refugia (Leathwick & Besier 2014). In this context, it is important that the recommended dose of a parasiticide is kept when treating animals. Underdosing might increase the chances of parasites to survive the treatment and to develop resistances (Koopmann 2008). For goats, there is a special situation, since currently no anthelmintic is authorised for their treatment. Veterinarians have to rededicate parasiticides, which are e.g. authorised for sheep, for the treatment of goats (Emmerich 2011). In these cases, dosage recommendations for sheep are often adopted for goats. However, as goats have a faster metabolism than sheep, they need a 1.5–2 times higher dosage. Due to such underdosing, few goats kept in a herd of sheep may lead to the development of resistances (Koopmann 2008).

In addition, treatment frequencies should be reduced to the minimum required to sufficiently control parasitoses, and strategically useful times of treatment should be chosen.

[4] *Dyf-7* is an extracellular matrix protein enabling neurite growth and maintenance by anchoring dendrites, e.g. during embryonic and larval development (http://www.uniprot.org/uniprot/Q09276).

If there are indications of a resistance towards a specific parasiticide, this parasiticide should no longer be used for antiparasitic treatments on this farm. Instead, an active pharmaceutical ingredient from another chemical group should be used. In most cases, an elimination of drug resistance cannot be expected, even if the respective parasiticide is not used for years. Generally, it is recommended to change the used parasiticide regularly in order to prevent the development of resistances (Deplazes et al. 2013), or to apply a combination treatment consisting of two parasiticides with different mechanisms of action (Leathwick et al. 2015).

An appropriate management of grazing land and biological control measures (see section 9.3) can also contribute to prevent resistances against parasiticides. Furthermore, care should be taken to avoid introducing resistant parasites into the herd when buying new animals. This can be ensured by quarantine measures and an appropriate treatment of the newly acquired animals.

In view of the current situation regarding resistances, modern methods to control parasites should not only be based on the use of parasiticides, but also include more complex and sustainable approaches (see section 9.3). As mentioned above maintaining refugia, in which susceptible parasites survive, is most important to prevent further development and distribution of parasitic resistances in the future.

7 Evaluation of excretion of commercially available avermectins and milbemycins by pasture animals

Information on the amount and time course of the excretion of administered parasiticides is relevant when discussing sustainable approaches to control parasites in pasture animals (section 9.3), and risk mitigation measures aiming at protecting soil (section 9.4.2) and, especially, dung organisms (section 9.4.1). Therefore, a search of the scientific literature was performed by the University of Hohenheim for excretion rates of avermectins and milbemycins, which are authorised for use in pasture animals (see section 2, Table 1). The aim was to collate detailed information on the faecal excretion of avermectins and milbemycins for relevant routes of application and animal species. For the present project, the most important data are the amount of excreted substance (relative to the applied dose), the time-point of maximum faecal excretion and the total duration of faecal excretion. A large part of the relevant data on faecal excretion was already published about 10 to 20 years ago. In addition to the scientific literature, approval documents and dossiers were screened for specific information on the abovementioned faecal excretion data. This included reports on maximum residue limits (MRL) of the European Medicines Agency (EMA), documents from the European Heads of Medicines Agencies (HMA), the U.S. Environmental Protection Agency (EPA), as well as environmental impact considerations of the U.S. Food and Drug Administration (FDA). These documents mainly include detailed information on the pharmacokinetics of the anthelmintics with plasma concentration profiles, plasma half-lives, target tissues, residue patterns in tissues and organs (i.e. maximum residue limits in liver, fat, muscle, milk, and kidney), and main excretion routes. However, data concerning the excretion profiles (e.g. concentrations in faeces over a time period, duration of excretion) are generally scarce in publically available dossiers. A comparison of dossier data to the data found in our evaluation of published scientific excretion data (Tables 11 – 17) reveals that the available dossier data largely reflect the information, which is also found in the scientific literature.

Overall, the identified data on the excreted amounts in the faeces of typical pasture animals (cattle, sheep and horses) are rather heterogeneous. Moreover, only limited data are available for some of the parasiticides considered within the present project. In the case of emamectin and selamectin, this is due to the fact that these two substances are especially used in plants and in companion animals, respectively (see Table 1). Excretion data often vary between different animal breeds (e.g. milk vs. meat breeds) and age classes (Hosking et al. 2010, Křížová-Forstová et al. 2011) as was also discussed at the project workshop (Annex 2). However, the available data are too limited to systematically evaluate this variability. Furthermore, there are inconsistencies in some of the publications, which complicate the interpretation of the excreted amounts and the time periods, during which the parasiticides were detected in faeces (see footnotes to Tables 13 – 19). Due to the relatively limited amount of data, excretion data for bolus application, were also included in the evaluation. However, it should be considered that avermectin containing boli are no longer approved in the EU. At present, only two boli containing benzimidazoles are approved for livestock in Germany (http://www.vetidata.de).

In pasture animals, avermectins are only marginally metabolized and mainly excreted via the faeces. The excretion rate via urine is below 2% (Kövecses & Marcogliese 2005). Moxidectin is metabolised to larger extent. As the avermectins, it is mainly excreted via the faeces (see Tables 16 and 17).

Generally, excretion depends on many factors including the animal species, breed, sex, body condition, age and physiological status as well as different factors regarding the application of the drug, especially the route of administration and the formulation of the drug (Gonzáles Canga et al. 2009, Křížová-Forstová et al. 2011; see also Annex 2).

The route of administration may influence both the excretion peak and the duration of the faecal excretion of avermectins and milbemycins. Several studies showed that both oral and parenteral application led to a higher peak of excretion of the parasiticide than topical application, while the duration of

excretion seemed to be shortened (e.g. Aksit et al. 2016). However, due to the factors mentioned above and the heterogeneity and relatively limited amount of the data, a general statement is not possible.

The most important results for ivermectin, doramectin, avermectin B_1, eprinomectin and moxidectin are summarised in the following sections. For more detailed information on the relatively heterogeneous excretion data, please refer to Tables 13 – 19.

Ivermectin

Independent from the route of application and animal species, ivermectin is mainly excreted via bile fluid and faeces (Campbell 1983, Sutherland & Campbell 1990, Scott & McKellar 1992, Steel 1993, Lanusse 1997).

In cattle treated by subcutaneous injection, approx. 62% of the dose is excreted via the faeces and 1.5% via urine within 7 days after treatment. Within the same period after intraruminal application, 80% are excreted via the faeces and 0.5% via urine (Steel 1993).

When ivermectin is applied orally to horses, the maximal concentrations in the faeces are reached approx. 2.5 days after application. Ivermectin can be detected in faeces as long as 40 days after application. However, about 90% of the applied dose is eliminated via faeces within 4 days after application (Pérez et al. 2001).

Feeding of the animals has an impact on the excretion of ivermectin via faeces. After a subcutaneous injection of ivermectin, cattle on the pasture additionally fed with hay had significantly lower ivermectin concentrations in the faeces (0.09 mg/kg [5]) than animals hold in a stable and fed with hay and lupine grain (0.36 mg/kg). This is due to the higher volume of faeces of the animals on the pasture (Cook et al. 1996).

Doramectin

Doramectin is mostly excreted via bile fluid and faeces (87%); only about 0.04% of the applied dose is eliminated via urine. In horses, the highest concentration of doramectin in the faeces is reached 24–48 h post application (Gokbulut et al. 2001). Although about 90% of the dose is eliminated within 10 days after application, doramectin can be detected in faeces as long as 100 days after application (Gokbulut et al. 2001).

Avermectin B_1 (abamectin)

Avermectin B_1 is excreted via sheep faeces for a long time: 70 days after single subcutaneous treatment with 0.2 mg/kg body weight, its concentration in faeces exceeded 200 µg/kg dry weight (Kožuh Eržen et al. 2005). The highest concentration in faeces (1277± 74 ng/g dry faeces) was detected 3 days after treatment. From day 4 to 9 onwards, the concentration in faeces decreased rapidly.

Eprinomectin

The main excretion of eprinomectin is via bile and faeces, while urine only contains small amounts of the parasiticide (Kožuh Eržen et al. 2007). In comparison to orally administered ivermectin, topically administered eprinomectin leads to a significantly lower peak concentration in faeces, while its persistence in the faeces is longer (Gokbulut et al. 2016). Subcutaneous application of eprinomectin tends to result in higher faecal peak concentrations but a shorter detection period in faeces than topical administration (Aksit et al. 2006). After topical application in cattle, the maximum concentration of eprino-

[5] Although this is not clearly stated by the authors, it can be assumed that the indicated ivermectin concentrations refer to the wet weight of the dung.

mectin in dung occurred 3 days after treatment, and eprinomectin could be detected in faeces for at least 29 days (Lumaret et al. 2005).

Moxidectin

Moxidectin is excreted mainly (> 90%) via the faeces (Ungemach 1994). After oral application to horses, the maximal concentrations in the faeces are reached approx. 2.5 days after application. About 90% of the dose is eliminated within 8 days after application, but moxidectin can be detected in faeces as long as 75 days after application (Pérez et al. 2001).

Detailed information on the excretion of avermectins and moxidectin by pasture animals is presented in Tables 11–17. In the following section, effects of these parasiticides on dung organisms are addressed.

Table 13: Excretion of avermectins by pasture animals following oral application

Active pharmaceutical ingredient	Trade name	Application: dose, route, season (where available)	Excretion of non-metabolised ingredient (% of the applied amount of active ingredient)	Time point of maximum excretion (days), maximum concentration	Duration of detection period, measured concentration	Analytical method: limit of quantification (LOQ), limit of detection (LOD)	Metabolites	Animal species (number), country	Reference
Ivermectin	Ivomec® SR Bolus (Merck & Co., Inc,)	1.72 g ivermectin as a bolus (12.7 mg are released daily for 135 d)	n.a.	d 14 during treatment: 4.0 ± 2.0 µg/g dry faeces (0.5 ± 0.2 µg/g wet faeces). Continuous excretion during 49 d	On d 49 (end of the study) still detectable: 3.0 ± 2.0 µg/g dry faeces (0,5 ± 0.4 µg/g wet faeces)	HPLC LOD and LOQ not specified	n.a.	Cattle (n=4) USA	Herd et al. 1996
Ivermectin	Ivomec® SR Bolus (MSD AGVET, France)	1.72 g ivermectin as a bolus (12.7 mg are released daily for 135 d)	80-90% excreted via faeces	d 4 during treatment: 4.1 µg/g wet faeces[6]. Steady-state concentration until d 120 (1.18 µg/g)	d 160 (end of the study): 2.67 ng/g wet faeces	HPLC LOQ: 0.5 ng/g LOD not specified	n.a.	Cattle (n=6) Argentina	Alvinerie et al. 1998
Ivermectin	Eqvalan® 1.87% w/v (Merck; Sharp & Dome Agvet)	0.2 mg/kg bw p.o.	74.3 ± 20.1%	d 2.5 p.a._ 2413 ± 1894 ng/g wet faeces	d 40: 0.6 ± 0.2 ng/g wet faeces d 50 not detectable	HPLC LOQ: 0.5 ng/g wet faeces LOD not specified	n.a.	Horse (n=5) France	Pérez et al. 2001
Ivermectin	Eqvalan® 1.87% w/v (Merck, USA)	0.2 mg/kg bw p.o.	n.a.	24 h: 19.5 µg/g dry faeces	After 120 h[7]: not detectable	HPLC LOQ 0.05 µg/g LOD not specified	n.a.	Horse (n=8), UK	Gokbulut et al. 2001

[6] Alvinerie et al. (1998): Controversial information in the discussion (peak concentration: 3.5 µg/g wet faeces on day 5)

[7] Gokbulut et al. (2001): unclear if 120 d or 120 h

Active pharmaceutical ingredient	Trade name	Application: dose, route, season (where available)	Excretion of non-metabolised ingredient (% of the applied amount of active ingredient)	Time point of maximum excretion (days), maximum concentration	Duration of detection period, measured concentration	Analytical method: limit of quantification (LOQ), limit of detection (LOD)	Metabolites	Animal species (number), country	Reference
Ivermectin	Noromectin Oral paste, 1.87% w/v	0.2 mg/kg bw p.o.	n.a.	7149 ng/g dry faeces	n.a.	HPLC with fluorescence detection	n.a.	Horses (n=5) UK	Gokbulut et al. 2016
Doramectin	Dectomax® (1% w/v, American Cyanamide, USA)	0.2 mg/kg bw p.o.	n.a.	24 h 20.5 µg/g dry faeces	After 120 h[8]: not detectable	HPLC LOQ: 0.05 µg/g LOD not specified	n.a.	Horse (n=8) UK	Gokbulut et al. 2001

Abbreviations: bw: body weight, p.a.: post application; p.o.: per os

[8] Gokbulut et al. (2001): unclear if 120 d or 120 h

Table 14: Excretion of avermectins by pasture animals following parenteral application

Active pharmaceutical ingredient	Trade name	Application: dose, route, season (where available)	Excretion of non-metabolised ingredient (% of the applied amount of ingredient)	Time point of maximum excretion (days) Maximum concentration	Duration of detection period, measured concentration	Analytical method: limit of quantification (LOQ), limit of detection (LOD)	Metabolites	Animal species (number), country	Reference
Avermectin B_1 (abamectin)	Abamitel® (L.A., Krka, Slovenia)	0.2 mg/kg bw s.c. June–September	n.a.	d 3 p.a.: 1277 ± 74 ng/g dry faeces	d 29 p.a.: not detectable	HPLC LOQ 2.5 ng/g LOD <1.0 ng/g dry faeces[9]	n.a.	Sheep (n=6) Slovenia	Kolar et al. 2006
Ivermectin	Ivomec® (Merck)	0.2 mg/kg bw s.c	n.a.	d 3 p.a.: 1.2 ± 0.3 µg/g dry faeces 0.2 ± 0.05 µg/g wet faeces	d 28 p.a.: 0.08 ± 0.0001 µg/g dry faeces, 0.01 ± 0.0008 µg/g wet faeces	HPLC LOD and LOQ not specified	n.a.	Cattle (n=4) USA	Herd et. al 1996
Ivermectin	Ivomec® (Merck Sharp and Dohme)	0.2 mg/kg bw s.c	35 ± 10%[10] within 31 d after treatment	d 5.6 ± 3.4 p.a.: 871.9 ng/g dry faeces	d 31 p.a.: 11.2 ± 11.8 ng/g dry faeces	HPLC LOQ: 5 ng/g dry faeces LOD: 2 ng/g dry faeces	n.a.	Cattle (n=5) Spain	Fernandez et al. 2009
Ivermectin	n.a.	0.2 mg/kg bw s.c	n.a.	d 2 p.a.: 3.9 ppm (µg/g dry faeces)	d 13.5 p.a.: 0.3 ppm (µg/g dry faeces)	HPLC LOD: 0.37 ng/ml (=0.05 ppm (µg/g dry faeces))	n.a.	Cattle (n=8) Denmark	Sommer & Steffansen 1993
Doramectin	1% solution	0.2 mg/kg bw s.c. January	n.a.	d 3 p.a.: 101 µg/kg wet faeces	d 42 p.a.: ≤ 20 µg/kg wet faeces	HPLC LOQ: 2.5 µg/g wet faeces LOD: not specified	n.a.	Cattle (n=50) Australia	Dadour et al. 2000

[9] Unclear (dry faeces vs. wet faeces); see also Kolar et al. (2006): dry faeces in the results; wet faeces in material and methods

[10] The quantity of ivermectin excreted in the dung within 31 d after application was 38.1 mg; total excretion obtained from the AUC_t was 34.4±10.1 mg

Active pharmaceutical ingredient	Trade name	Application: dose, route, season (where available)	Excretion of non-metabolised ingredient (% of the applied amount of ingredient)	Time point of maximum excretion (days) Maximum concentration	Duration of detection period, measured concentration	Analytical method: limit of quantification (LOQ), limit of detection (LOD)	Metabolites	Animal species (number), country	Reference
Doramectin	Dectomax® (Pfizer, France)	0.2 mg/kg bw s.c. June–September	n.a.	d 2 p.a.: 2186 ± 145 ng/g dry faeces	d 36 p.a.: 4.9 ± 2.1 ng/g dry faeces d 42: not detectable	HPLC LOQ: 2.5 ng/g dry faeces[11] LOD < 1.0 ng/g dry faeces	n.a.	Sheep (n=6) Slovenia	Kolar et al. 2006
Eprinomectin	n.a.	0.2 mg/kg bw s.c.	n.a.	223 ng/g dry faeces	0.8-13.6 d p.a.	HPLC with fluorescence detection	n.a.	Dairy cattle (n=5) UK	Aksit et al. 2016

Abbreviations: bw: body weight, p.a.: post application; s.c.: subcutaneous injection.

[11] Unclear if dry faeces or wet faeces: wet faeces are mentioned in material and methods, dry faeces in the results.

Table 15: Excretion of avermectins by pasture animals following topical application

Active pharmaceutical ingredient	Trade name	Application: dose, route, season (where available)	Excretion of non-metabolised ingredient (% of the applied amount of ingredient)	Time point of maximum excretion (days) Maximum concentration	Duration of detection period, measured concentration	Analytical method: limit of quantification (LOQ), limit of detection (LOD)	Metabolites	Animal species (number), country	Reference
Ivermectin	n.a.	0.5 mg/kg bw pour-on	n.a.	d 1 p.a. 9.0 ppm (µg/g dry faeces)	d 13 p.a. not detectable	HPLC LOD 0.37 ng/ml (=0.05 ppm [µg/g] dry faeces)	n.a.	Cattle (n=8) Denmark	Sommer & Steffansen 1993
	Noromectin® (Norbrook, UK)	0.5 mg/kg bw pour-on April-June	n.a.	d 1 p.a. 21.9 mg/kg dry faeces	d 47 p.a. < 3.0 mg/kg dry faeces	HPLC LOD and LOQ not specified	n.a.	Cattle (n=10) UK	Sutton et al. 2014
	Ivomec® (pour-on bovine; Merial)	0.5 mg/kg bw pour-on	n.a.	d 3 p.a. 0.78 µg/g wet faeces	Detectable until d 12	HPLC LOQ: 5 ng/g wet faeces LOD not specified	n.a.	Cattle (n=2) France	Bousquet-Mélou et al. 2004
	Ivomec®	0.5 mg/kg bw pour-on	n.a.	d 2 p.a.: 18.5 ± 7.4 µg/g dry faeces, 2.8 ± 1.2 µg/g wet faeces	d 28 p.a. 0.04 ± 0.004 µg/g dry faeces 0006 ± 0.0004 µg/g wet faeces	HPLC LOD and LOQ not specified	n.a.	Cattle (n=4) USA	Herd et al. 1996
Doramectin	Dectomax® (pour-on, Pfizer Santé Animale)	0.5 mg/kg bw pour-on	n.a.	d 5 p.a.: 0.45 µg/g wet faeces	d 33 p.a. 0.04 µg/g wet faeces	HPLC LOQ: 5 ng/g wet faeces LOD: not specified	n.a.	Cattle (n=2) France	Bousquet-Mélou et al. 2004
	[^{3}H] Doramectin	0.5 mg/kg bw pour-on	Until d 56 p.a.: approx. 30%	d 21 p.a.: 156 ng/g[12] for females, 270 ng/g for males	d 56 p.a.: 7.4 ng/g in females, 3.9 ng/g in males	Radiotracer analysis (LSC, HPLC) LOD and LOQ not specified	approx. 10% of the radiotracer: O-desmethyl doramectin	Cattle (n=4) USA	US FDA 2002

[12] Unclear if concentrations indicated in US FDA (2002) relate to dry or wet faeces.

Active pharmaceutical ingredient	Trade name	Application: dose, route, season (where available)	Excretion of non-metabolised ingredient (% of the applied amount of ingredient)	Time point of maximum excretion (days) Maximum concentration	Duration of detection period, measured concentration	Analytical method: limit of quantification (LOQ), limit of detection (LOD)	Metabolites	Animal species (number), country	Reference
Eprinomectin	Eprinex® pour-on	0.5 mg/kg bw pour-on	20.5% ± 4.3[13]	d 3 p.a.: 0.35 ± 0.22 µg/g wet faeces	d 29 0.004 ± 0.005 µg/g wet faeces	HPLC LOD: 1 mg/g wet faeces[14] LOQ: not specified	n.a.	Cattle (n=5) France	Lumaret et al. 2005
Eprinomectin	Eprinex®	1 ml/10 kg bw	n.a.	d 2 p.a.	d 14	HPLC	n.a.	Cattle (n=6)	Halley et al. 2005
	n.a.	pour-on	n.a.	0.427 mg/kg wet faeces	0.00185 mg/kg wet faeces detectable	LOQ: 0.0036 mg/kg	n.a.	USA	Kožuh Eržen et al. 2007
	n.a.	0.5 mg/kg bw	n.a.	3.34 mg/kg dry faeces	d 32 p.a.	LOD: 0.0018 mg/kg	n.a.	Sheep (n=6)	Aksit et al., 2016
	Eprinex® pour-on	pour-on	n.a.	d 3 p.a.	4.8 ng/g dry faeces	HPLC	n.a.	Slovenia	Gokbulut et al. 2016

Abbreviations: bw: body weight, p.a.: post application

[13] No calculation is provided by Lumaret et al. 2005

[14] Discrepancy between the LOD and the eprinomectin concentrations determined in dung

Table 16: Excretion of milbemycins by pasture animals following oral application

Active pharmaceutical ingredient	Trade name	Application: dose, route, season (where available)	Excretion of non-metabolised ingredient (% of the applied amount of ingredient)	Time point of maximum excretion (days) Maximum concentration	Duration of detection period, measured concentration	Analytical method: limit of quantification (LOQ), limit of detection (LOD)	Metabolites	Animal species (number), country	Reference
Moxidectin	Eqvest® (2% gel)	0.4 mg/kg bw per os	44 ± 18%	d 2.5 p.a.: 2594 ±1234 ng/g wet faeces	d 75 p.a.: 4.3 ± 2.8 ng/g wet faeces	HPLC LOQ: 0.5 ng/g wet faeces LOD not specified	n.a.	Horse (n=5) France	Pérez et al. 2001
	Equine gel, 2% w/v (Pfizer Inc, USA)	0.2 mg/kg bw per os	n.a.	24 h: 16.6 µg/g dry faeces	120 h[15]: not detectable	HPLC LOQ: 0.05 µg/g dry faeces LOD not specified	n.a.	Horse (n=8) UK	Gokbulut et al. 2001

Abbreviations: bw: body weight, p.a.: post application

[15] Gokbulut et al. (2001): unclear if 120 d or 120 h

Table 17: Excretion of milbemycins by pasture animals following parenteral application.

Active pharmaceutical ingredient	Trade name	Application: dose, route, season (where available)	Excretion of non-metabolised ingredient (% of the applied amount of ingredient)	Time point of maximum excretion (days) Maximum concentration	Duration of detection period, measured concentration	Analytical method: limit of quantification (LOQ), limit of detection (LOD)	Metabolites	Animal species (number), country	Reference
Moxidectin	^{14}C and ^{3}H labelled moxidectin	0.2 mg/kg bw s.c.	d 28 p.a. 58% in faeces 3% in urine	d 3 p.a. = peak of excretion (5–8% of administered dose) from d 4 p.a. onwards: 1–3% of administered dose per day	n.a.	HPLC LOQ/LOD not specified	Faeces: on d 2 p.a. 74% and on d 7 p.a. 78% of detected residues were metabolites. Major metabolite (25–34%): a monohydroxy metabolite (at C-29 or on side chain)[16]. Urine: on d 1 p.a. 99.9% of excreted residues were metabolites (dihydroxy-metabolites)[17]	Cattle (n=3) USA	Zulalian et. al. 1994
Moxidectin	^{14}C and ^{3}H labelled moxidectin	0.2 mg/kg bw s.c.	d 7 p.a.: 52% of total administered radioactive moxidectin detect-	n.a.	n.a.	HPLC	2 metabolites detected (hydroxylated moxidectin	Sheep (n=12) USA	Afzal et al. 1994

[16] Other metabolites were minor (<10%); dihydroxy metabolite (CH_2OH at C-14 and OH on side chain; CH_2OH at C-14 or C-24 and OH on side chain) or monohydroxy metabolite with CH_2OH at C-14.

[17] Major metabolites were (a) 48% dihydroxy metabolite with CH_2OH at C-14 and OH on side chain, (b) 10.3% dihydroxy metabolite with CH_2OH at C-14 or C-24 and OH on side chain and (c) 13.9% not further specified.

Active pharmaceutical ingredient	Trade name	Application: dose, route, season (where available)	Excretion of non-metabolised ingredient (% of the applied amount of ingredient)	Time point of maximum excretion (days) Maximum concentration	Duration of detection period, measured concentration	Analytical method: limit of quantification (LOQ), limit of detection (LOD)	Metabolites	Animal species (number), country	Reference
			ed in faeces and < 1% in urine				and 23-keto derivate of moxidectin)		

Abbreviations: bw: body weight, p.a.: post application; s.c.: subcutaneous

Table 18: Excretion of milbemycins by pasture animals following topical application

Active pharmaceutical ingredient	Trade name	Application: dose, route, season (where available)	Excretion of non-metabolised ingredient (% of the applied amount of ingredient)	Time point of maximum excretion (days) Maximum concentration	Duration of detection period, measured concentration	Analytical method: limit of quantification (LOQ), limit of detection (LOD)	Metabolites	Animal species (number), country	Reference
Moxidectin	Cydectine® 0.5% (Fort Dodge Santé Animale)	0.5 mg/kg bw pour-on	n.a.	d 5 p.a.: 0.45 µg/g wet faeces	d 12 p.a.: moxidectin detectable in wet faeces at low level	HPLC LOQ 0.05 ng/g wet faeces LOD not specified	n.a.	Cattle (n=2) France	Bousquet-Mélou et al. 2004
Moxidectin	Cydectine® pour-on 10% (Fort Dodge Animal Health, USA)	0.5 mg/kg bw pour-on May – July	n.a.	d 3 p.a.: 0.95 µg/g wet faeces and 0.49 µg/g wet faeces in two independent trials	d 21 p.a.: not detectable d 28 p.a.: not detectable in two independent trials	HPLC LOQ, LOD not specified	n.a.	Cattle (n=5 in first trial, n=6 in second trial) Japan	Iwasa et al. 2008

Abbreviations: bw: body weight, p.a.: post application

Table 19: Comparison of excretion data

Active pharmaceutical ingredient	Animal species	Excretion in %	Major metabolites	Reference
Eprinomectin	Calves	17-99% of administered dose were excreted via faeces, 82-87% were excreted as non-metabolised residue	24a-hydroxymethyl metabolite	EMEA/CVMP 1996 Boxall et al. 2002
Ivermectin	Cattle, sheep	63-98% of administered dose were excreted via faeces, 39-78% were excreted as non-metabolised residue	24-hydroxymethyl-H_2B_1a; 3-O-desmethyl-H_2B_1a; 3-O-desmethyl-H_2B_1b	CVMP 1998 Boxall et al. 2002

8 Effects of avermectins and milbemycins on dung organisms (dung flies and beetles)

Since the results of ecotoxicological tests with dung organisms were compiled quite recently (Lumaret et al. 2012), the current evaluation by ECT mainly focused on studies published in the last five years. Different search terms, most importantly the names of the compounds as well as further keywords, such as dung organism*, dung beetle*, dung flies, ecotoxic* and effect, were used. This search (using e.g. Scopus and ScienceDirect) revealed about ten further relevant publications, in particular the reviews of Beynon (2012) and Jacobs & Scholtz (2015). Finally, own recent work regarding the ecotoxicological effects of parasiticides has been added.

It should be noted that only in newer publications (since about 2005) effects are given as concentrations of the test substance in dung (e.g. as mg/kg dung fresh weight (fw) or dung dry weight (dw)). These concentrations are measured via residue analysis. In view of the fact that before 2005, no legal requirements for effect values (NOEC, LOEC, EC_{50}) such as VICH (2005) existed, the aim of most older studies was to identify for how long after treatment the dung of farm animals was toxic to dung organisms. In these papers, effects are usually expressed as days or weeks after treatment (DAT and WAT). Very rarely, concentrations of the parasiticides were measured in parallel.

8.1 Effects of avermectins on dung organisms

Avermectin B_1 (abamectin)

The available information on the ecotoxicological effects of avermectin B_1 is summarised in Table 20. Besides data compiled by Lumaret et al. (2012) no other papers were identified. Effect concentrations were only derived in very few studies. In fact, only an EC_{100} of ≥16 µg a.i./kg is available that was determined in a non-standard beetle test. Substantial effects on several endpoints were found both for beetles and flies starting in dung sampled 3–7 DAT and lasting up to 8 WAT.

Table 20: Summary of ecotoxicological data from tests with avermectin B_1 and dung organisms

Test organism	Exposure	Endpoint	Effect	Reference
Dung beetles (Coleoptera)				
Digitonthophagus gazella	Injectable 200 µg/kg bw (cattle)	Larval mortality Oviposition	100% at ≥ 16 µg/kg fw No effect at 16 µg/kg fw	Doherty et al. 1994
Onthophagus binodis	Injectable 200 µg/kg bw (cattle)	Larval survival Oviposition	Reduced for 4–8 WAT	Ridsdill-Smith 1988
	Injectable 200 µg/kg bw (cattle)	Survival of newly emerged beetles	Reduced in dung 3–6 DAT	Houlding et al. 1991
	Injectable 200 µg/kg bw (cattle)	Survival of newly emerged beetles Egg laying in dung voided	Reduced in dung 3–6 DAT Inhibition for 5–6 WAT	Dadour et al. 2000
Dung flies (Diptera)				
Musca vetustissima	Injectable 200 µg/kg bw (cattle)	Egg-adult mortality Egg-adult mortality	100% in dung up to DAT 14 2% in dung at WAT 8	Ridsdill-Smith 1988
M. vetustissima	Injectable	Survival	Reduced at WAT 4; no	Clarke & Ridsdill-

Test organism	Exposure	Endpoint	Effect	Reference
	200 µg/kg bw (cattle)	Asymmetry of wing veins	effect at WAT 8–11	Smith 1990
	Injectable 200 µg/kg bw (cattle)	Larval survival	0% at DAT 3–25 6% at DAT 35	Wardhaugh & Mahon 1991
	Injectable 200 µg/kg bw (cattle)	Fly survival	Suppressed for 16–32 DAT	Wardhaugh & Mahon 1998

Abbreviations: DAT: days after treatment, WAT: weeks after treatment, bw = body weight

Doramectin

In addition to the data compiled by Lumaret et al. (2012), no further publications on the toxicity of doramectin to dung organisms were identified. In the field, doramectin seems to cause an increase in wing asymmetry in the dung fly *Scathophaga stercoraria*, while no effects on the abundance of these flies has been found (Webb et al. 2007). The available information indicates that doramectin is highly toxic, especially for dung beetles (Table 21).

Table 21: Summary of ecotoxicological data from tests with doramectin and dung organisms. All effect values refer to nominal concentrations

Test organism	Exposure	Endpoint	Effect	Reference
Dung beetles (Coleoptera)				
Aphodius sp.	Pour-on 500 µg/kg bw (cattle)	Colonisation of dung pats in the field	Preference for control pats	Webb et al. 2010
Onthophagus binodis	Injectable 200 µg/kg bw (cattle), fed to beetles	Survival of newly emerged beetles Egg laying and adult survival	Reduced in dung for 9 DAT Reduced on DAT 3 and DAT 6	Dadour et al. 2000
Digitonthophagus gazella	n.a.	Mortality	LC_{50} 12.5 µg/kg fw LC_{90} 38.2 µg/kg fw	Boxall et al. 2002
Dung flies (Diptera)				
Musca domestica	Pour-on 500 µg/kg bw (cattle)	Larval emergence	None at WAT 1, less than in control 2 and 4 WAT	Floate et al. 2001
Musca inferior	Pour-on 500 µg/kg bw (cattle)	Larval survival	Reduced in dung for 9-13 DAT	Wardhaugh et al. 2001

Abbreviations: bw = body weight, DAT: days after treatment, WAT: weeks after treatment

Emamectin

Neither in the review of Lumaret et al. (2012) nor in the web information on the ecotoxicological effects of emamectin on dung organisms was found. In fact, it seems that only butterfly species (Lepidoptera) have been tested so far. In these studies, a contact method was used, in which the test substance was dissolved in water, meaning that this exposure scenario is by no means comparable to the exposure in dung. However, it is possible that some data were overlooked, since in the web search several papers were listed, but could not be evaluated as they were written in Vietnamese.

Eprinomectin

Additionally to the data provided by Lumaret et al. (2012), only one publication was identified (Iwasa & Sugitani 2014). Ecotoxicological studies with eprinomectin are rare compared to those with ivermectin or moxidectin. Hence, the toxicity of eprinomectin is difficult to evaluate. The few available data (Table 22) indicate a medium toxicity for dung flies and no long-lasting toxicity to dung beetles, but this statement is based on a very weak data basis.

Table 22: Summary of ecotoxicological data from laboratory tests with eprinomectin and dung organisms. All effect concentrations refer to nominal concentrations

Test organism	Exposure	Endpoint	Effect	Reference
Dung beetles (Coleoptera)				
Onthophagus taurus	Pour-on 500 µg/kg bw (cattle)	Larval mortality	High for 1–2 WAT	Wardhaugh et al. 2001
Caccobius jessoensis	Pour-on 500 µg/kg bw (cattle)	Adult emergence	Significant reduction at DAT 1–3	Iwasa & Sugitani 2014
Liatongus minutus	Pour-on 500 µg/kg bw (cattle)	Larval survival	Significant reduction at DAT 1–3	Iwasa & Sugitani 2014
Dung flies (Diptera)				
Musca inferior	Pour-on 500 µg/kg bw (cattle)	Larval mortality	Increased for 9-13 DAT	Wardhaugh et al. 2001
Haematobia inferior	Pour-on 500 µg/kg bw (cattle)	Larval emergence	None at WAT 1, reduced at WAT 2 and 4	Floate et al. 2001
Neomyia cornicina	Pour-on 500 µg/kg bw (cattle)	Pupation and emergence rate	Hampered on DAT 1 and 3	Iwasa & Sugitani 2014
Neomyia cornicina	Pour-on 500 µg/kg bw (cattle)	Emergence Larval mortality	None until DAT 12 NOEC: 7 ± 5 µg/kg fw (concentration on d 20) High until DAT 12	Lumaret et al. 2005

Abbreviations: DAT: days after treatment, WAT: weeks after treatment, bw = body weight

Ivermectin

In Table 23, the most relevant information on the ecotoxicological effects of ivermectin is summarised. This compound is surely the best studied veterinary pharmaceutical in terms of side-effects on dung organisms. Direct sublethal effects of ivermectin on dung insects, i.e. their physiology and behaviour, have been identified, e.g. in the beetle *Scarabaeus cicatricosus*, even at concentrations lower than those determined in dung (Verdú et al. 2015). A detailed assessment of its environmental risks has been published by Liebig et al. (2010). The information provided in this assessment and in the compilation of Lumaret et al. (2012) consists of 85 data sets. In the present contribution, the above-mentioned reviews and three additional studies (Cruz Rosales et al. 2012, Blanckenhorn et al. 2013a, b) were considered. Blanckenhorn et al. (2013b) did not only test the six species listed in Table 21 but also further 19 species of the family Sepsidae from all over the world. The LC_{50} values determined in these tests differed by a factor of up to 370.

Actually, ivermectin is the only substance considered in this section that has been assessed on the basis of standardised OECD tests, which have been adopted less than 10 years ago (OECD 2008, 2009). These data (partly gained in international ring tests, e.g. Römbke et al. 2010a) are among the most sensitive test results so far. In the only study reporting even lower effect concentrations (0.5 µg/kg, Strong & James 1993), fluctuating asymmetry of morphological traits of the yellow dung fly *Scathophaga stercoraria* was used. These observations could not be confirmed later on (Floate & Coghlin 2010).

Various field studies confirm the effects of ivermectin on dung organisms and – regularly but not always – on dung degradation under relevant usage conditions (e.g. Römbke et al. 2010b, Sutton et al. 2014). In general, dung flies seem to be more affected than dung beetles, but this situation is complex, e.g. because of the attraction of ivermectin (or possibly solvents) on adult dung beetles.

In addition, the relationship between functional and structural effects on the dung organism community is still not well understood as was also discussed at the project workshop (see summary in Annex 2). Surely, local conditions (e.g. climate, occurrence of non-arthropod organism affecting dung degradation such as earthworms) also have to be considered. For example, no adverse effect of ivermectin administration on the abundance, species richness or species diversity of dung beetles could be identified in a field monitoring study in Central Japan (Imura et al. 2014). Climatic factors as well as soil properties or cattle grazing intensity influence the abundance and diversity of dung beetle communities. Comparable experiences regarding the different sensitivity of dung beetles were made when comparing the effects of ivermectin at three sites in Europe: less effects were observed in Southern France compared to The Netherlands (Römbke et al. 2016). However, in the same project less effects of ivermectin were also found at a site in Switzerland, indicating that climate is not the only factor to be considered here.

Table 23: Summary of ecotoxicological data from laboratory and field tests with ivermectin and dung organisms. Only selected results (those providing detailed and sensitive data) are included. All effect concentrations refer to nominal concentrations

Test organism	Exposure	Endpoint	Effect	Reference
Dung beetles (Coleoptera)				
Aphodius constans	Spiked dung (cattle) OECD standard test	Larval mortality after 21 d	LC_{50} 176 µg/kg fw LC_{50} 880 µg/kg dw NOEC 320 µg/kg dw	Hempel et al. 2006
	Pour-on 500 µg/kg bw (cattle)	Larval mortality after 21 d	LC_{50} 100 µg/kg fw LC_{50} 590 µg/kg dw	Lumaret et al. 2007
Euoniticellus intermedius	Spiked dung	Developmental time Larval emergence	Reduction at 10 µg/kg fw None at 1 mg/kg fw	Cruz Rosales et al. 2012
Volinus distinctus	Injectable 200 µg/kg bw (cattle)	Abundance in the field	EC_{50} 0.62 mg/kg dw NOEC 0.5 mg/kg dw	Römbke et al. 2010b
Dung flies (Diptera)				
Musca autumnalis	Spiked dung (cattle) OECD standard test	Emergence rate after 21 d	EC_{50} 4.65 ± 2.17 µg/kg fw NOEC 1.1–3.3 µg/kg fw	Römbke et al. 2010a
	Spiked dung	Mortality	LC_{50} 4.65 µg/kg fw LC_{50} 33.1 µg/kg dw	Blanckenhorn et al. 2013a
Musca domestica	Spiked dung	Mortality	LC_{50} 24.7 µg/kg fw LC_{50} 176.2 µg/kg dw	Blanckenhorn et al. 2013a
Scathophaga stercoraria	n.a.	Larval mortality after 48 h Developmental time (3–4 weeks)	LC_{50} 36 µg/kg fw EC_{50} 1.0 µg/kg fw	Strong & James 1993
	Spiked dung (cattle) OECD standard test	Larval mortality after 28 d Developmental time after 28 d	LC_{50} 20.9 µg/kg fw NOEC <0.84 µg/kg fw	Römbke et al. 2009
	Spiked dung	Larval mortality	LC_{50} 20.9 µg/kg fw LC_{50} 149.0 µg/kg dw	Blanckenhorn et al. 2013a
Scathophaga suilla	Spiked dung	Larval mortality	LC_{50} 8.84 µg/kg fw LC_{50} 63.0 µg/kg dw	Blanckenhorn et al. 2013a
Sepsis duplicata	Spiked dung	Larval mortality	LC_{50} 0.09 µg/kg fw LC_{50} 0.64 µg/kg dw	Blanckenhorn et al. 2013a
Sepsis neocynipsea	Spiked dung	Larval mortality	LC_{50} 0.232 µg/kg fw LC_{50} 1.65 µg/kg dw	Blanckenhorn et al. 2013a
Dung fly larvae (community)	Injectable 200 µg/kg bw (cattle)	Abundance in the field	NOEC< 0.31 mg/kg dw	Römbke et al. 2010b

Abbreviations: fw: fresh weight, dw: dry weight, DAT: days after treatment, WAT: weeks after treatment

Selamectin

Neither in the review of Lumaret et al. (2012) nor in our literature search information on the effects of selamectin on dung organisms was found.

8.2 Effects of milbemycins on dung organisms

Moxidectin

Only one publication (Blanckenhorn et al. 2013a) was identified in addition to the data compiled by Lumaret et al. (2012). In Table 24, only selected results (those providing detailed and sensitive data) are listed. Please note that besides the listed effect concentrations for the most sensitive fly species *Sepsis neocynipsea*, similar values for seven other species of the family Sepsidae (Diptera) are available – all of them compiled in one publication (Blanckenhorn et al. 2013a). The sensitivity of these species, sampled in various parts of the world, differs by a factor of up to 300.

Table 24: Summary of ecotoxicological laboratory data from tests with moxidectin and dung organisms. Only selected results (those providing detailed and sensitive data) are included. All effect concentrations refer to nominal concentrations

Test organism	Exposure	Endpoint	Effect	Reference
Dung beetles (Coleoptera)				
Aphodius sp.	Injectable (cattle)	Larval survival	LC_{50} 4.0–5.4 mg/kg dw LC_{50} 0.60–0.81 mg/kg fw	Hempel et al. (2006)
Digitonthophagus gazella	Injectable (cattle)	Reproduction	EC_{50} 0.256 mg/kg fw	Fort Dodge Animal Health (1997)
Euoniticellus intermedius	Injectable (cattle)	Adult mortality Reproduction	NOEC > 0.50 mg/kg fw EC_{50} 0.47 mg/kg fw NOEC >0.27 mg/kg fw	Fort Dodge Animal Health (1997)
Dung flies (Diptera)				
Musca autumnalis	Spiked dung	Mortality	LC_{50} 0.07 mg/kg fw LC_{50} 0.47 mg/kg dw	Blanckenhorn et al. 2013a
Musca domestica	Spiked dung	Larval mortality	LC_{50} 0.14 mg/kg fw LC_{50} 0.92 mg/kg dw	Blanckenhorn et al. 2013a
Neomyia cornicina	Pour-on (cattle)	Egg-adult survival	EC_{50} 0.06 mg/kg fw (at 7 DAT)	Iwasa et al. 2008
Scathophaga stercoraria	Spiked dung	Larval mortality	LC_{50} 0.12 mg/kg fw LC_{50} 0.80 mg/kg dw	Blanckenhorn et al. 2013a
Scathophaga suilla	Spiked dung	Larval mortality	LC_{50} 0.09 mg/kg fw LC_{50} 0.58 mg/kg dw	Blanckenhorn et al. 2013a
Sepsis neocynipsea	Spiked dung	Larval mortality	LC_{50} 0.01 mg/kg fw LC_{50} 0.08 mg/kg dw	Blanckenhorn et al. 2013a

Abbreviations: fw: fresh weight, dw: dry weight, DAT: days after treatment

Milbemycinoxim

Neither in the review of Lumaret et al. (2012) nor in our literature search information on the ecotoxicological effects of milbemycinoxim on dung organisms was found. This result is not surprising, since this compound is generally not used for farm animals but for pets.

8.3 Summary: effects of avermectins and milbemycins on dung organisms

Looking at the data compiled in this report the following conclusions can be drawn, taking into consideration that only for ivermectin a robust data set (including field results) is available):

- Usually, flies are reacting more sensitively than beetles.
- Reproductive endpoints are more sensitive than mortality, but often not by a large margin. This can be explained by the fact that in many tests with mortality/survival as endpoint not adults but larval stages are used and that these stages are reacting very sensitively.

Still, the toxicity of the considered parasiticides to dung organisms differs, both in terms of effect concentrations and the duration of significant effects. However, it is difficult to compare the toxicities of the parasiticides, because the number of available data differ considerably. As mentioned before, ivermectin is the best-studied compound, followed by moxidectin. Information for the other substances is scarce. The toxicity of ivermectin, avermectin B_1, doramectin, eprinomectin and moxidectin to dung flies is very high. Where available, L/EC_{50} values are lower than 10 µg/kg fw; effect durations are ranging from several days up to several weeks. In contrast, L/EC_{50} values for dung beetles range from <16 µg/kg fw for avermectin B_1 and doramectin to 100 and 256 µg/kg fw for ivermectin and moxidectin, respectively, with eprinomectin in the middle.

Several authors point out that not only in the laboratory but, more importantly, in field studies moxidectin is less toxic for dung organisms than other parasiticides such as ivermectin or doramectin (e.g. Floate et al. 2002, Iwasa et al. 2008, Suárez et al. 2009). However, such comparisons between different parasiticides have rarely been performed within one study, meaning that results from different sites, climatic conditions, organism communities etc. have to be compared. As an exception proving the rule, Floate (2007) performed a three-year-comparative field study in Canada, using four parasiticides (doramectin, eprinomectin, ivermectin, moxidectin). His results partly confirmed the outcome of a previous literature review (Floate 2006) based on Canadian data. He concludes that the toxicity of these four substances can be classified as follows: doramectin is the most toxic and moxidectin the least toxic substance for dung organisms, while ivermectin and eprinomectin are of intermediate and, more or less, similar toxicity. However, this classification might not be relevant everywhere. For instance, effects of doramectin and ivermectin on dung organism communities could be quite similar, e.g. in Argentinian grasslands (Suárez et al. 2003). Both environmental conditions as well as the composition of the respective dung organism community has to be taken into account here.

In summary, the data compiled in the present project are in line with classifications from Floate (2006, 2007). However, this statement is hampered by the fact that the dataset used by Floate (2007) and the dataset used here are overlapping and that the number of available data for the four parasiticides is differing considerably. No conclusions can be drawn regarding the toxicity of emamectin, selamectin and milbemycinoxim to dung organisms, since data are very scarce and, partly, even non-existing.

Until quite recently, it was very difficult to understand why several organism groups, mainly arthropods but also nematodes, react very sensitively to avermectins while other groups do not. According to Puniamoorthy et al. (2014) the sensitivity of individual dung organism groups depends at least partly on ancient phylogenetic patterns, meaning that Ecdysozoa (i.e. moulting animals such as nematoid worms or insects) are more susceptible to avermectins than other organism groups, for example, annelid worms. This knowledge might be helpful in developing new parasiticides with less side-effects to non-target organisms.

In the present project, no attempt was made to cover the increasing number of publications addressing the ecological role of dung organisms and their contribution to ecosystem functioning, mainly the role of species-rich dung beetle communities in buffering ecosystem services in perturbed agro-ecosystems (e.g. Beynon et al. 2012a, b; Manning et al. 2016, Verdú et al. 2017). However, it should be pointed out that dung organism communities are one of the best examples how to value ecological

functions and services – especially when they are affected by chemical substances. In this context it should be mentioned that field studies would be helpful in order to evaluate not only direct effects of the tested parasiticides on dung organisms, but also the consequences of such effects in terms of functional (e.g. dung degradation) or structural (e.g. biodiversity) endpoints. Based on the considerable amount of information on such effects in the field (e.g. Lumaret et al. 2012, Römbke et al. 2017) EMA recently prepared a draft guideline for such field studies (EMA/CVMP 2016b).

9 Evaluation of risk management strategies for parasiticides used in pasture animals

9.1 Definitions

In the present project, the term 'risk management strategies' is used for all strategies aiming at a reduction of the environmental risk caused by veterinary pharmaceuticals (or, more specifically, antiparasitics). This includes sustainable approaches to control parasites (section 9.3), risk mitigation measures (section 9.4) and restrictions of use (section 9.4.3).

Risk mitigation measures (RMMs) are concrete measures that are identified and implemented during the authorisation process of a veterinary pharmaceutical to reduce the environmental risk caused by this product to an acceptable level (see e.g. de Knecht et al. 2009).

The term 'manure' is used for both, liquid and solid excretions of animals, which may be mixed with other materials (e.g. straw; Junker et al. 2016).

Liquid manure is a mixture of liquid and solid excretions of animals and water that was used to clean the stables. It has a high liquid content, and is generally collected in storage tanks. Liquid manure may also contain residual bedding material (e.g. straw). The typical dry matter contents of liquid cattle manure is approx. 10% (Junker et al. 2016).

The term 'dung' is used for solid excretions of animals (faeces).

9.2 Background

In environmental risk assessments submitted during the authorisation process of avermectins and milbemycins, high environmental risks for dung organisms and aquatic organisms were identified. Despite this fact, the products containing these parasiticides were authorised. In the summaries of product characteristics (SPC) for these products, risk mitigation measures are described, which aim at reducing the environmental risk caused by the respective product (Adler et al. 2016a). In this context, it should also be mentioned that the inclusion of RMMs in the summaries of product characteristics (SPCs) is compulsory, if a potential risk is identified. However, the implementation of these RMMs is not legally binding and compliance is not monitored (i.e. there are no sanctions in case of non-compliance; see also Annex 2).

Possible RMMs are discussed within each authorisation process. In this context, it has often been criticised that the measures are not feasible with regard to the agricultural practice. According to EMA/CVMP (2012) RMMs, which a substantial number of farmers cannot comply with, are not appropriate. However, Liebig et al. (2011, 2014) have suggested a more differentiated approach, since even if a measure can only be applied under certain conditions (depending e.g. on the farming method), this RMM may still contribute considerably to reduce the environmental risk.

Risk management strategies (including RMMs) are of particular relevance for parasiticides that are persistent, bioaccumulative and toxic [18]. Based on a preliminary screening, a number of widely used parasiticides has been identified as potential PBT substances. One of these substances, moxidectin, fulfils the criteria for PBT classification (EMA/CVMP 2016a). According to EMA/CVMP (2015, 2016a), veterinary medicinal products containing an active pharmaceutical agent with PBT properties should

[18] Substances that are persistent, bioaccumulative and toxic (PBT) are of specific concern, and their identification is part of various regulations (see e.g. Moermond et al. 2012). Within the environmental risk assessment of veterinary pharmaceuticals, a PBT screening is performed for the active substances (EMEA/CVMP 2008, EMA/CVMP 2015). Based on EMA/CVMP (2015) the PBT assessment should be performed according to Annex XIII of the REACH Directive (EC 2011) and REACH guidance R.11 (ECHA 2012). However, so far it is not clear how PBT properties should be considered in the benefit/risk evaluation of veterinary pharmaceuticals, and which consequences a classification of the active substance as PBT might have.

only be authorised if (1) the potential for exposure of the environment is limited (e.g. because the active pharmaceutical agent is extensively metabolised to non-PBT substances), (2) the risk can be adequately controlled using effective risk mitigation measures, (3) no suitable alternative products (without PBT properties) or technologies are available, or (4) the therapeutic benefits clearly outweigh the environmental risk.

Within the present project, risk management strategies (sustainable approaches to control parasites, risk mitigation measures and restrictions of use) for parasiticides used to treat pasture animals (cattle, sheep and horses) were compiled and discussed. The focus is placed on ivermectin, doramectin, eprinomectin and moxidectin (see section 2), i.e. parasiticides fulfilling some or, in case of moxidectin, all PBT criteria (see also Table 33, section 9.4.3).

9.3 Sustainable approaches to control parasites

In conventional animal farming, a frequent application of parasiticides to all animals without relation to the actual parasitic burden (i.e. a strategic treatment) was common for a long time. In this approach, the intervals between treatments during the first half of the grazing season are usually related to the time until reappearance of parasite eggs or larvae, which depends on the parasite species and the grazing stage (e.g. approx. 5 weeks in cattle, 8–12 weeks in horses). If this practice is used, a large amount of parasiticides is applied, which at least partly end up in the environment, and the risk of resistance development is increasing. However, due to the resistance situation, the treatment regimes have often – depending on the animal species and indication – been modified and treatment frequencies reduced as was also emphasized at the project workshop (see section 9.3.1 and Annex 2).

The following approaches to avoid these problems and to increase the sustainability of antiparasitic treatments were compiled and discussed by the University of Hohenheim: (1) to optimise treatment regimes, (2) to improve grazing land management, (3) to optimise animal husbandry practices, and (4) to employ alternative control measures (sections 9.3.1–9.3.4). In this context, it has to be pointed out that the situation is very diverse. It involves the treatment of various animal species, breeds and age classes, a number of different parasites (see section 5) differing in their developmental cycles, various epidemiological situations, several parasiticides and application forms (sections 2 and 5) and different farming methods / husbandry systems. As a result, approaches have to be case-specific. Due to the complexity of the situation, it is not sufficient to only consider the active substance, the application form and the animal species. In the following subsections, general issues will be outlined.

9.3.1 Optimised treatment regimes

Infections of ruminants, such as cattle and sheep, and horses with parasites are a common cause of reduced health and performance (e.g. weight loss and reduced growth) in young animals, while in approx. 70–80% of the older animals the course of infestations with parasites is often clinically inapparent, controlled by the immune system and connected to a low parasite burden. This means that in the latter case, an antiparasitic treatment does not seem to be necessary, because there is a balance between the immune system of the host and the low parasitic load. However, especially non-immune young animals, which are for the first time on the pasture, are threatened by parasites and require treatment as was also stressed at the project workshop.

In view of already existing resistances and, especially, to avoid the development of new resistances it is essential to reduce the treatment with antiparasitics to the minimum, which is required to sufficiently control parasitoses, and to combine the treatment with appropriate livestock management measures. To reduce the necessity of anthelmintic treatments, the infection pressure has to be minimised. In the following, different possibilities to reduce or prevent new infestations with parasites are discussed. As many measures as possible should be applied before treating the animals with an an-

thelmintic (prudent usage of anthelmintics). Furthermore, the success of the treatment with an anthelmintic should be evaluated regularly using e.g. an egg count reduction test (Janssen 2013).

A strategic treatment with an antiparasitic should only be applied, if an infestation worth treating has been diagnosed and the treatment has been prescribed by a veterinarian (Heckendorn & Frutschi 2014). This veterinary prescription is regulated in the German Medical Product Law (Arzneimittelgesetz) for certain medicinal products including those intended for use in food-producing animals. If possible, a selective treatment (e.g. a selective deworming) or a targeted selective treatment should instead be used, i.e. only a part of the herd should be treated, while the other animals should remain untreated (Koopmann 2008, Bauer 2015).

In a selective treatment, the animals to be treated are chosen more or less independently from their actual parasite burden. For instance, a certain age group or a certain percentage of animals (e.g. 30%) is treated. A selective treatment can be carried out with or without concomitant parasitic diagnosis within the herd. If a selective instead of a strategic treatment is applied, a reduced amount of the antiparasitic is used, the selective pressure on the parasite is lowered and the abovementioned refugia are created.

In a targeted selective treatment, individual animals are treated. The selection of these animals is mainly based on the number of eggs excreted per gram of faeces, but can also be based on other signs indicating a parasitic infestation. Thus, the minority of animals that is responsible for the majority of excreted eggs is treated, while the untreated immune animals serve as refugia for dung fauna and parasites supporting a reduced selection of resistant parasites.

So far, selective and targeted selective treatment approaches are mainly practiced for horses and adult cattle, but are rarely used for sheep. Yet, principally these approaches are suitable for all three pasture animal species (see e.g. Kenyon & Jackson 2012, Charlier et al. 2014, Scheuerle et al. 2016).

The success of all selective treatment approaches depends on the training and information of the farmers, a good communication between veterinarians and farmers as well as an appropriate clinical, epidemiological, and diagnostic evaluation of each specific situation.

The greatest challenge is to identify the animals that have to be treated (Koopmann 2008) as was also stressed at the project workshop. This can be done using faecal examinations (see above). Faecal egg counts represent a good decision tool at herd level. Still, when selecting the most affected animals it has to be considered that the amount of excreted eggs is not always correlating with the actual parasite burden. This was shown by comparing the faecal egg count and the post mortem count of nematodes in the intestinal tract of individual animals. Moreover, the excretion of eggs by parasites is not continuous. Hence, it is not always possible to predict the actual parasite burden of single animals by enumerating the eggs excreted per gram of faeces (Deplazes et al. 2013) [19]. This problem can be solved by collecting faeces on several days and/or from several animals in a group to obtain adequate information on the infection load in this group. Another disadvantage of this method is the lack of automation and, thus, the relatively high effort and high costs for herd diagnostics (see below). Further approaches include clinical examinations of the animals (such as the FAMACHA© scheme for *Haemonchus* infections in sheep) and an evaluation of performance parameters, such as milk production in cattle and body condition (Kleinschmidt 2009). However, there is need for further research to improve the diagnostics, especially with regard to practical and cost-effective methods, which can easily be applied in extensive cattle and sheep farming.

Targeted selective treatment cannot be performed with young animals, e.g. horses younger than three years, not even if they show low numbers of eggs per gram of faeces. Due to their insufficiently developed immune system, they have to be dewormed strategically at specific intervals (Samson-Himmelstjerna et al. 2011, Bauer 2015).

[19] It should also be noted that the number of worms does not necessarily correlate with clinical disease (

Treatment strategies that are based on laboratory diagnoses are, of course, associated with costs (between 8 and 25 € per sample). However, they have several clear advantages: (1) the number of treatments and, accordingly, the amount of used parasiticides is reduced, (2) the amount of parasiticides excreted by the treated animals to grassland is lowered, and (3) the selection pressure on the parasites is reduced. Within the untreated animals, so-called refugia develop, where parasites are not subjected to a selective pressure. These refugia are of great relevance to avoid the development of resistances (see section 6).

In summary, selective treatment approaches essentially depend on the possibilities to identify the animals, which have to be treated, and to select the optimal time-points for diagnosis and therapy. There is still a need for research on indicators, which can be used to decide if a treatment is required and when this treatment should be performed (see also section Annex 2).

Apart from the number and the selection of the animals that should be treated, the time and place of application of a parasiticide could be important with regard to possible environmental risks and the development of antiparasitic resistances. If animals, which are not kept on pastures throughout the whole year, are treated at least 3–5 days before being turned out to pasture (rather than on the day when being turned out to pasture, or later during the pasture season), the amount of the parasiticide excreted to the pasture would be reduced. However, as discussed at the project workshop (see Annex 2), the animals (especially first year grazing animals) have to be treated when infection pressure is high. From a veterinary perspective, first year grazing cattle should e.g. be treated e.g. 6–8 weeks after the start of the grazing period depending on the worm species. Hence, the possibilities to shift treatment times in order to reduce the amount of parasiticides excreted to the pasture appear limited. A detailed analysis is required for each farm animal species, parasite and antiparasitic product. Due to the complex interactions of parasite biology, infection pressure (esp. on pastures), prevalence of the parasitosis in the herd, herd anamnesis, resistance situation and availability of approved antiparasitics, such an analysis should be performed by farmers, veterinarians, parasitologists, and environmental experts in close cooperation.

The selection of a route of application that results in a shorter duration of excretion might be relevant, if pasture animals are kept in stables during and shortly after treatment (see above and section 9.4.1.3). For instance, it may be possible to limit the duration of excretion of a parasiticide by the chosen application route (oral or parenteral instead of topical; see section 7). Which route of application is most appropriate has to be evaluated for each animal species, breed and age group, the specific parasiticide and the used formulation.

However, such an evaluation is only possible for cattle, where a range of approved products with different routes of administration and the relevant excretion data are available. Doramectin is approved as parenteral and topical application to cattle, eprinomectin as topical, and ivermectin and moxidectin as parenteral and topical application (see section 2, Table 1). For doramectin, the peak of excretion in faeces is on day 3 (parenteral application) or on day 5 (topical), for eprinomectin on days 2–3 (topical), for ivermectin on days 2–9 (parenteral) or days 1–3 (topical), and for moxidectin on day 3 (parenteral) or on day 3–5 (topical) (see section 7).

For horses, only the peroral application of avermectins and milbemycins is approved. In sheep, avermectins (ivermectin and doramectin) are only approved for parenteral application, moxidectin for peroral application (Table 1). The excretion data identified within the present project for sheep are limited to the excretion of doramectin after parenteral application (section 7, Table 14).

In this context, it should be mentioned that an optimised treatment also means that the recommended dose of a parasiticide is kept when treating animals and that additional recommendations for preventing resistances are considered (see section 6).

An important point concerning the prevention or at least delay of the development of resistances in nematodes is the preservation of sufficiently large refugia, where all developmental stages of the para-

sites are not in contact with anthelmintic drugs and, therefore, are not subjected to selective pressure. If these refugia are available, the parasite population consists of a majority of untreated and therefore sensitive individuals, and only a few resistant nematodes that have survived an anthelmintic treatment. In such a population, resistance alleles cannot propagate rapidly, and the resistance development is delayed (Kleinschmidt 2009). This can be achieved using selective treatments or targeted selective treatments as outlined above.

Treatments with parasiticides should be scheduled, i.e. they have to be epidemiologically justified and performed at certain times to cause a lasting disruption of the developmental cycle of the parasite (Deplazes et al. 2013). To this aim, detailed information on the parasites, which are actually present in the animals, on the pastures and in the farms, is essential.

However, it should never be the aim of a treatment plan to eliminate all parasites. A low infection pressure is desirable, because it leads to the development of a protective immunity within the animals. In most cases, animals with an induced immune response hardly show symptoms or a reduction in performance because of a parasitic infection. Consequently, a lower amount of parasiticides has to be used to treat animals with a protective immunity. By contrast, young grazing animals (especially during their first grazing period) react very sensitively to parasitic infestations (Heckendorn & Frutschi 2014) and need appropriate treatment with anthelmintic drugs.

Formerly, treatment of the whole herd on a pasture was recommended before switching pastures. By doing so, the contamination of the new pasture with worm eggs should be kept to a minimum. However, this so-called 'dose and move strategy' showed to promote the development of resistances, since resistant worms that had survived the treatment, formed almost the whole population on the new grazing land. Without competition with susceptible worms, the resistant individuals are able to propagate rapidly (Koopmann 2008). Therefore, this strategy cannot be recommended.

In summary, the selection of the most effective and sustainable deworming concept is rather challenging, given the complex nature of the parasites and the different options to control parasites in the different animal species, husbandry and pasture systems. Valuable tools have been developed that help to select a suitable strategy for the specific situation and farm (e.g. http://www.weide-parasiten.de).

In view of the future success and sustainability of antiparasitic treatment, it would be extremely useful to obtain monitoring data on the prevalence of parasites in farms, the usage of parasiticides, the success of antiparasitic treatments, and the resistance situation in parasites (see also workshop protocol in Annex 2 of this report). The data that have to be compiled for the different livestock animal species should include information on the farming system, used antiparasitic treatment strategies, and environmental factors (e.g. climatic conditions such as temperature and humidity).

Based on such data, recommendations could be provided to farmers how to control parasitoses combining optimised antiparasitic treatments and further approaches to control the parasites, similarly as for antibiotics in the information platform Aniplus (https://www.aniplus.de).

9.3.2 Management of grazing land

Successful parasite control starts with an appropriate management of grazing lands. Within Germany, there are considerable regional differences in pasture management. Some of the proposed measures are already implemented in agricultural practice. However, is has to be pointed out that a successful management of all pasture-, parasite-, and animal-related processes (i.e. the combination of all measures) is decisive for a successful control of parasitoses and a reduction of the use of antiparasitics. Since pastures, livestock animals and parasites are affected by rainfall and other weather conditions, and are subjected to seasonal cycles and natural life cycles, these factors have to be considered in the overall management strategy.

Based on the life cycle of each parasite, management measures should be applied that are – with few exceptions – equally suitable for cattle, small ruminants and horses (Deinhofer 2009). If at least some preventive measures against parasites are implemented, the frequency of antiparasitic treatments can be reduced, and the resistance and residue problems can be alleviated.

In springtime, before animals are out to pasture, different parasite developmental stages, which have survived the winter, can be found on the grazing land. This overwintering population is of particular relevance, as it can infect naïve animals and develop into patent infections that contribute significantly to the overall infection pressure. With the rising temperature, the grass begins to grow and the larvae become more active. Over the following weeks, the measurable density of larvae declines continuously. On the one hand, this is caused by the growing vegetation leading to a 'dilution' of the larvae. On the other hand, larvae that are weakened by the winter die. The shorter the grass is at the beginning of the grazing season, the higher is the number of infectious larvae ingested with the grass. This means that from a parasitological perspective, animals should be out for grazing as late as possible (Prosl 2009). However, animal welfare and practical considerations limit the practicability of this recommendation. Another possible measure to reduce the number of ingested larvae is to use the first grass grown in spring to produce hay or silage. By doing this, the infectious larvae are removed and the majority of them is inactivated by drying up or by the reduction of the pH during the production of silage. Drying of the soil and the influence of UV light additionally reduce the number of larvae on the pasture.

A further possibility for reducing the number of parasite larvae on a pasture in springtime is not to use the whole pasture the year before. This measure is already in use. The area that is not used can be cut during summer or autumn, so that the parasite stages are removed (Hinney 2012). This area can be used for grazing in the next spring. Using grazing land for hay or silage production before or after being grazed is generally advantageous.

If animals are kept on the pasture during daytime and in sheds over the night, bringing them out too early in the morning should be avoided. Most parasite larvae gather in areas of high humidity (e.g. in dewdrops on grass). When animals graze in the early morning, they can ingest a huge number of larvae. For animals that are on pasture during day and night, the ingestion of larvae can be reduced by additionally feeding hay in the morning (Deinhofer 2009). After rainfall, infectious larvae move out of the faeces and gather in the water film on the grass (Prosl 2009). Here, the same measures may be applied as mentioned above: (1) no use of the pasture until it is dry, and (2) if the pasture is used continuously, additional feeding of hay to reduce the number of ingested larvae. However, the acceptability and practicability of these measures may be limited, e.g. due to the increased workload.

As helminth larvae prefer a warm and humid environment, humid areas should be avoided on pastures. Wet ground beneath dripping water drums, creeks or wells are ideal biotopes for larvae and some intermediate hosts. Lymnaeid snails, which are intermediate hosts of liver flukes, can be found in any humid area. Liver fluke infections can easily be avoided by removing the snail's habitats (Deinhofer 2009). Rivers and creeks should be fenced, watering places should be kept dry, and depressed, humid areas on a pasture should preferably be filled up. Again, the increased workload may lead to a limited acceptability of these measures.

An appropriate stocking density on grazing land is important to control parasitic infestations and to reduce the antiparasitic treatment frequency. A stocking density that is too high results in a high contamination with parasite eggs and, consequently, an increased infection pressure. Additionally, the sward is grazed too low, i.e. almost all larvae are ingested by the animals (Deinhofer 2009). For instance, a stocking density of 0.5 animals/ha is recommended for horses (Berndgen 2002). An optimised stocking density is also relevant in view of good agricultural practice, and good animal health and performance.

Rotational grazing systems with pastures divided in several plots, which are used subsequently, are a further option to protect grazing animals from parasitic infestations. The plots should have a size providing food for one or two weeks. After this period, the animals are moved to the next plot. Plots already used for grazing should not be used again for the same animal species for at least 4–6 weeks. During summer, the majority of parasites will die due to dryness and UV light. In an ideal situation, each plot is used for grazing only once a year, and afterwards for the production of hay and/or silage. However, this approach requires a sufficiently large area of grazing land and is relatively time-consuming, e.g. since the plots have to be fenced in with electric wire.

Grazing of different animal species on the same pasture is another opportunity to reduce the parasite pressure. Appropriate species (see below) ingest the parasite larvae as dead-end hosts. In these hosts, the parasites cannot develop and no eggs are excreted, i.e. dead-end hosts function as a sort of 'vacuum cleaner'. This measure is suitable for selected parasites, if cattle and small ruminants graze on the same pasture, while other parasites can harm both species (Deplazes et al. 2013). In our highly specialized animal husbandries, this measure is rarely used.

Due to their immature immune status, young animals are generally more sensitive to parasite infestations than older animals. Therefore, the parasite pressure has to be kept very low on pastures for young animals. Young animals should preferably spend their first grazing season with older animals on dry pastures with a low stocking density, and they should be moved regularly to a new pasture or plot. If only young animals are kept on a pasture, the parasite density increases rapidly, since due to their low immunity the young animals are perfect hosts for parasites. In mixed cattle herds, the adult animals ingest the majority of larvae and protect the young animals against an excessive infestation. To ensure that the young animals acquire a sufficient basic immunity, a grazing period of 4–5 months is required, during which the young animals are in contact with the parasites (Heckendorn & Frutschi 2014).

Collecting dung (faeces) is a very effective measure to reduce the parasite pressure on paddocks (Samson-Himmelstjerna 2013). Due the texture and size of the dung, this measure is mainly suitable for horses, but generally not practicable for cattle and small ruminants such as sheep. Ideally, dung should be collected at least twice a week. Several studies show that this approach prevents the spreading of nematodes on paddocks more effectively than the use of anthelmintic drugs (Corbett et al. 2014). When the dung is collected, infectious larvae are removed from the paddock. If the dung is stored correctly, the larvae are inactivated. In case of dry weather, the paddocks can also be scrubbed. Scrubbing spreads the dung equally, so that it dries and infectious larvae are inactivated. However, scrubbing is not effective under humid weather conditions, because larvae are then spread over the entire paddock, but not inactivated.

Places, where animals often defecate and which are not used for grazing anymore, should be cut (Samson-Himmelstjerna 2013).

As mentioned at the project workshop (see Annex 2), pastures could be disinfected with calcium cyanamide to minimise parasitic stages. However, this measure is not validated yet, and preliminary data are inconsistent. Further research is needed to clarify if and how this disinfection could be performed successfully avoiding negative side-effects in the environment.

9.3.3 Optimised husbandry systems

The hygiene measures that are discussed in the following contribute to control the infection rate with gastrointestinal parasites. Since such measures can only be successful, if both pastures and stables are included, measures that are relevant for stabled animals are also considered.

When buying new animals, it has to be kept in mind that these animals could introduce gastrointestinal parasites (which may, in some cases, even be resistant to parasiticides) into the stock. Therefore, these animals have to be quarantined. When their parasite status is clear and, if necessary, the new animals have been treated successfully, they can be integrated into the herd. In this way, it can be avoided to import new parasite strains (including resistant ones; see section 6). Especially in small ruminants and horses, drug-resistant helminths are spreading because of livestock trade and common pastures for different stocks (Deplazes et al. 2013).

Stables and fenced runs should be kept clean and hygienic. Regular removal of the dung (see section 9.3.2), cleaning or disinfection partly removes exogenous parasites and, thus, reduces infestations (Hiepe et al. 2006, Samson-Himmelstjerna et al. 2011).

When feeding the animals in the stable, it is not only important to provide food of optimal quality and with a well-balanced nutrient content, but also to ensure that the food does not get in touch with the animal excretions (Deplazes et al. 2013). Fodder racks and elevated mangers can be helpful.

Animal excretions should be kept in appropriate storage systems as long as possible, since infectious larvae are inactivated during the composting processes (Hiepe et al. 2006; Samson-Himmelstjerna et al. 2011). A sufficiently long storage of liquid and solid manure also favours the degradation of parasiticides and, thus, reduces their entry into the environment (Lutz & Alber 2004; see also section 9.4.6).

9.3.4 Alternative control measures

There are several approaches for non-medical measures that may contribute to control parasitic infestations in animal farming. However, these approaches are still in an early developmental stage, and additional research is required before a possible application in agricultural practice. Nevertheless, these approaches are briefly presented in the following.

Nematophagous fungi are discussed as biological control measure against parasitic worms. These fungi live on gastrointestinal worm larvae. Fungal spores are fed to grazing animals. They survive the gastrointestinal passage and are excreted with the faeces. The spores germinate, and the nematophagous fungi live on the worm larvae in the faeces. Consequently, only few larvae reach the grass and the infection pressure is lowered significantly. So far, this measure has only been used in Brazil. However, it should be kept in mind as a potential measure to reduce infestations with helminths (Schnieder 2004, Assis et al. 2012, Heckendorn & Frutschi 2014).

The breeding of animals with an increased resistance to parasites could be a further option to biologically control parasites. In merino sheep, it has been possible to select animals with a higher resistance against abomasal parasites [20](e.g. *Haemonchus* spp.). These sheep show a lower rate of egg excretion and a lower morbidity (Deplazes et al. 2013). Studies on cattle spending their first summer on pastures also showed differences in the excretion of parasite eggs. These differences can partly be attributed to a genetically determined ability of the animals to control the excretion of eggs. When selecting for genetic resistance, animals showing a low egg excretion are chosen. However, one disadvantage of this method is that egg excretion and worm burden do not correlate very well (Schnieder 2004; see also section 9.3.1). In addition, breeding programs are always hampered by the fact that the selection for one trait (in this case resistance against parasites) can be associated with adverse effects on other traits including performance characteristics. As mentioned at the project workshop, it can be

[20] Parasites inhabiting as adults the fourth and last division of the stomach in ruminant animals.

assumed that resistance breeding programmes will become more important in future. In other countries, such breeding programmes already exist (e.g. in Switzerland, for sheep). It is important to note that such programmes need public funding (see also Annex 2).

Breeding resistant vectors is a further measure aiming at disturbing the development of certain parasites in a way that important vectors are no longer susceptible for the developing states of the parasites, but has so far not been used to control parasites in pasture animals (Deplazes et al. 2013).

The development of prophylactic immunization might be a further approach. For example, cattle could be vaccinated to provide protection against the lungworm *Dictyocaulus viviparus*. For this vaccination, irradiated infectious larvae are generally used (Hiepe et al. 2006). These irradiated larvae retain their immunogenicity, but their virulence and their ability to develop in the host are reduced in a way that they are no longer pathogenic. With slight adaptations of the irradiation dose, this measure is principally suitable for almost all helminths (Deplazes et al. 2013). Other vaccines used e.g. in Australia include a vaccine against *Haemonchus contortus*, which leads to an 80% reduction of egg excretion in vaccinated lambs (Strube & Daugschies 2015). Yet, no anthelmintic vaccine is currently available in Germany as was stressed at the project workshop. Several studies were performed to develop inactivated vaccines (subunit vaccines, antigens, native and recombinant inactivated vaccines, nucleic acids), but the success of these vaccinations compared to living vaccines is so far not satisfying (Deplazes et al. 2013). Successful vaccine development is hindered by several factors including very complex host-pathogen interactions during the course of parasitoses, which are still not fully understood. In summary, vaccines directed against nematodes are currently no option in Europe, and this situation is unlikely to change in the near future (Strube & Daugschies 2015).

Plants with certain healing effects, including plants helping to treat worm infections, have been known for a long time (Hiepe et al. 2006). Several food plants are supposed to have antiparasitic properties. They contain enzymes, alkaloids, glycosides or tannins, which are assumed to reduce the worm burden (Deplazes et al. 2013). Bloomfell, chicory, alpine sainfoin and quebracho are some of these plants, which are believed to have anti-parasitic properties (Podstatzky 2009). Yet, information on possible anthelmintic effects of specific plants is controversial: while some studies showed a reduction of the numbers of gastrointestinal nematodes when feeding bioactive plants, others did not (Podstatzky 2009, Deplazes et al. 2013). Due to anti-nutritive properties of some of these plants, they can only be fed in limited amounts (Heckendorn 2006, Deplazes et al. 2013). In addition, some plants used for the extraction of bioactive substances are toxic.

The use of condensed tannins seems to be a promising option to reduce nematode infestations in grazing animals, and research on this topic should be intensified. To date, it has not been clarified if the tannins have a direct effect on the nematodes or an indirect favourable effect on the gastrointestinal milieu. However, at present the mentioned plant products are no practicable alternative to anthelmintics.

In summary, it can be stated that there are some possible alternative measures that might contribute to control parasites in pasture animals. However, further research efforts are required with regard to all these measures. In addition, it should be pointed out that these approaches can only be part (complementary prophylaxis) of an integrated treatment programme, which also relies on anthelmintics as central component.

9.4 Risk mitigation measures

In a first step, risk mitigation measures for pasture animals were compiled by ECT based on EM(E)A documents (EMEA/CVMP 2008, EMA/CVMP 2012, 2015, 2016a) and the results of previous projects (Liebig et al. 2011, 2014, Vidaurre et al. 2016). A supplementary literature search using Scopus did not yield any further relevant publications. The identified RMMs were presented at the third project meeting (April 27th, 2016), and six RMMs were selected for evaluation and discussion. The selected RMMs were discussed by Fh-IME (section 9.4.2.1) and ECT (sections 9.4.1, 9.4.2.2 and 0).

Criteria that should be fulfilled by risk mitigation measures are indicated in EMEA/CVMP (2008). Several additional criteria were identified by Liebig et al. (2011, 2014; see Table 25).

Table 25: Criteria for evaluating risk mitigation measures (RMMs)

Criterion	Explanation	Reference
Efficacy	The RMM leads to a reduction of the environmental risk (generally by reducing exposure of the environment to the parasiticide)	EMEA/ CVMP 2008
Practicability	The RMM is feasible with respect to agricultural practice	
Legitimacy	The RMM is consistent with relevant regulations in the EU and its member states	
Verifiability	A verification of the effect of the RMM should be possible, e.g. by deriving a revised PEC taking the RMM into account	
Sustainability	The RMM has a long-lasting effect	Liebig et al. 2011, 2014
Proportionality principle	The RMM is (a) suitable to achieve the aim (i.e. to reduce the environmental risk), (b) the mildest measure to achieve this objective and (c) reasonable	
Addressing	The RMM is explicitly directed to the appropriate addressee	

The criteria 'efficacy' and 'practicability' were considered as far as possible when discussing the risk mitigation measures. However, in many cases data gaps were identified that have to be filled in order to specify the respective RMM. For instance, the present knowledge on the biology and ecology of dung organisms is insufficient to identify appropriate time windows, during which parasiticides could be used without harming dung organism communities (see section 9.4.1.1). Half-live times in manure are required to specify how long manure must be stored prior to spreading onto land (cf. section 9.4.2.1). Each RMM has to be specified before a detailed evaluation according to all criteria indicated in Table 25 can be made. It should be pointed out that such a detailed evaluation has to be performed for each parasiticide product, farm animal species and farming system. Further details such as the appropriate time of treatment for each farm animal species and parasite have to be considered where relevant. Within the present project, such a detailed evaluation was not feasible. This is due to the following reasons:

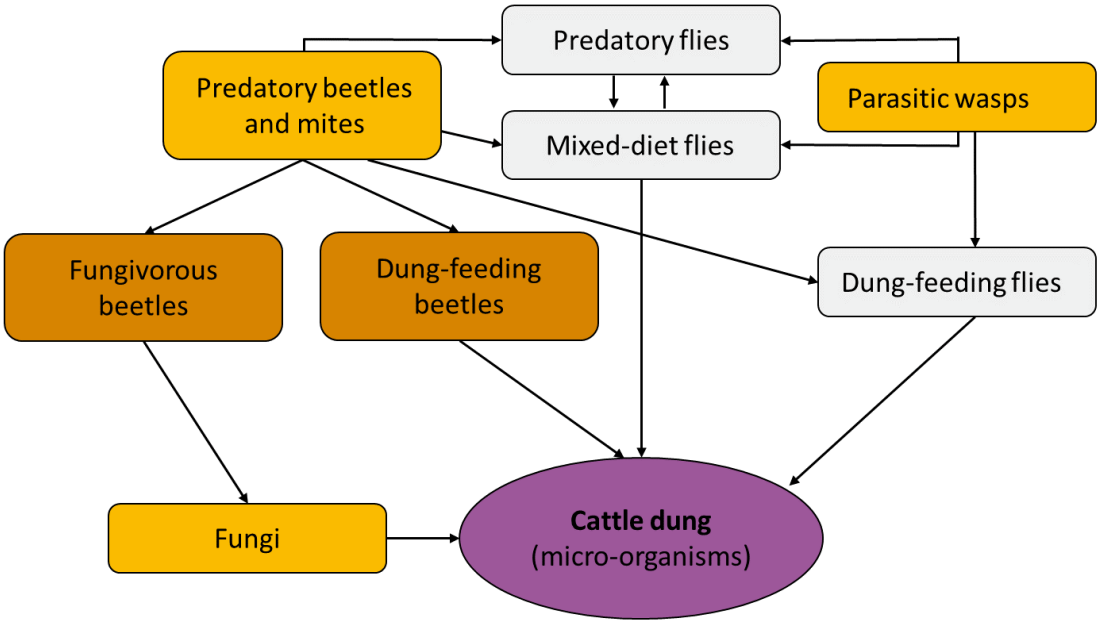
a) The overall situation is very diverse involving the treatment of different livestock animal species, breeds and age classes, various parasites differing in their developmental cycles, different epidemiological situations, several parasiticides and application forms and different farming methods / husbandry systems (see section 9.3).
b) Monitoring data on the prevalence of parasites in the different livestock animal species, the usage of parasiticides and the success of antiparasitic treatments (and treatment strategies) for different farming / husbandry systems are not available (see section 9.3.1).
c) Many risk mitigation measures cannot be sufficiently specified, since relevant data are lacking as outlined above (in sections 9.4.1 and 9.4.2, these data gaps are specified for the respective RMMs).

Proposals how to improve the current situation are made in sections 10 and 11.

9.4.1 Risk mitigation measures focusing on the protection of the dung organism community, i.e. their diversity and functions

In order to understand the possibilities and challenges of the following three RMMs, it is necessary to know the specific characteristics of dung ecosystems and the inhabiting organisms. First, a dung pat is really an ecosystem of its own, being inhabited by a very complex and species-rich community of organisms (Jochmann et al. 1991). A single dung pat may contain tens to hundreds of coprophilous arthropod species (insects and mites) exceeding 1000 individuals (Mohr 1943). Dung organisms can be defined as those species that spend part or all of their life in close association with dung pats, upon which they are reliant for breeding sites and/or as a source of food. This includes dung-feeding species (coprophagous beetles and flies) as well as their predators and parasitoids. Saprophagous species (e.g. earthworms, springtails, nematodes) are also part of this group. Although they are not reliant on dung, they are common in dung at later stages of decomposition. Collectively, these organisms form a highly complex, temporally and spatially variable community (Adler et al. 2016b). Dung pat communities are mainly comprised of arthropod guilds that are characterized by differences in their diet (Figure 21).

Figure 21: Interactions among arthropods and other organisms common in cattle dung



Presentation based on Floate et al. (2005), modified.

Following deposition of a fresh dung pat, flies quickly arrive to feed, mate and lay eggs that will develop into adults in just a few weeks (Hanski & Cambefort 1991). This first 'wave' of colonists is followed by the arrival of dung-feeding beetles, whose numbers will peak about one week after deposition. They can be classified into three ecological groups: dwellers, tunnelers, and rollers. Dung beetle species have longer generation times than flies, comprising several weeks to months. Parasitic wasps, gamasid mites and predaceous beetles feed on immature stages of dung beetles and flies. In total, all these processes occur within the first month after deposition (often less), and end when the dung has either formed a firm crust or when it is degraded. The feeding and breeding activities of these dung-dwelling species accelerate the process of dung degradation. In addition, birds (looking for prey) and/or farm animals (trotting through dung pats) can physically break up dung pats. The whole process is completed by soil organisms – mainly oligochaete earthworms and enchytraeid worms, but also springtails (Collembola) – feeding on the remaining dung from below the pats (Swift et al. 1979). Depending on the climatic region and especially soil moisture, earthworms may be more relevant for dung degrada-

tion than coprophilous dung flies and beetles (Holter 1979). In general, earthworms tend to be more important where present (i.e. in moist and cool regions), whereas insects dominate in dry and warm regions (Lumaret & Errouissi 2002).

For parasiticides used in pasture animals, possible risks to dung organisms are evaluated in the environmental risk assessment (VICH 2005, EMEA/CVMP 2008). Initial predicted environmental concentrations ($PEC_{dung\ initial}$) of the active substance in dung are calculated using the total residue approach, while excretion data are taken into account when deriving refined PEC values ($PEC_{dung\ refined}$). In phase II tier A of the risk assessment, predicted no effect concentrations (PNECs) for dung organisms are obtained by dividing the results of laboratory tests by a safety factor of 100. In phase II tier B, the effects on dung organisms and on the functional endpoint dung decomposition may be investigated in a field study. An example for PEC and PNEC values and the resulting risk quotients is given in Table 26 for ivermectin based on data from Liebig et al. (2010). Although the risk quotients decrease with increasing realism of the environmental risk assessment, a risk is identified both in phase II tier A and tier B.

Table 26: Predicted environmental concentrations (PECs) for ivermectin in dung, predicted no effect concentrations (PNECs) and no observed effect concentrations (NOECs) for ivermectin and dung organisms, and resulting risk quotients (based on Liebig et al. 2010)

	PEC_{dung} (µg/kg dung fw)	PNEC (µg/kg dung fw)	Risk quotients
Phase II tier A	$PEC_{dung\ initial}$ 4,800 – 12,700 [a, b]	Dung flies 0.047 [e]	103,000 – 273,000
		Dung beetles 1.76 [f]	2,700 – 7,200
	$PEC_{dung\ refined}$ 159 – 420 [a, c]	Dung flies 0.047 [e]	3,400 – 9,000
		Dung beetles 1.76 [f]	90 – 240
	PEC_{dung} (µg/kg dung dw)	**NOEC (µg/kg dung dw)**	**Risk quotients**
Phase II tier B	$PEC_{dung\ refined,\ beef\ cattle}$ 946 – 2365 [d]	Dung flies < 310 [g]	> 3.1 > 7.6
		Dung decomposition < 780 [g]	> 1.2 > 3.0

[a] Initial and refined PECs were derived according to EMEA/CVMP (2008) for various ivermectin-containing products for different livestock animal species and ages (beef cattle, sheep: adult ewe and lamb, horse, and pony) using information on dosages (0.2–0.5 mg/kg bw) and application frequencies as given in the respective summaries of product characteristics (Liebig et al. 2010).

[b] Initial PECs were derived using the total residue approach (Liebig et al. 2010).

[c] Refined PECs were calculated using the highest fraction of the applied dose excreted in one day (3.31%, see Liebig et al. 2010).

[d] Refined PECs for beef cattle were calculated as described in footnotes a and c (Liebig et al. 2010, Liebig et al., unpublished data).

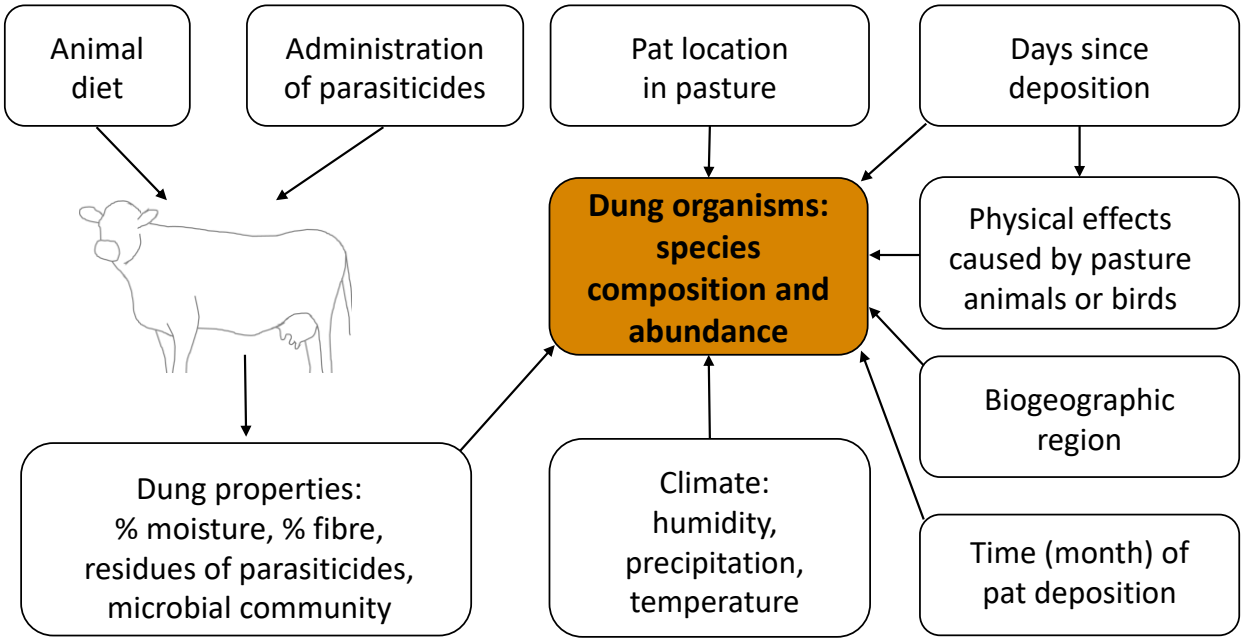
[e] In a 21-d test with the dung fly *Musca autumnalis*, an EC_{50} of 4.65 µg/kg dung fw was derived for emergence rate (Römbke et al. 2010a). The PNEC was derived by dividing this EC_{50} by a safety factor of 100 (Liebig et al. 2010).

[f] In a 21-d test with the dung beetle *Aphodius constans*, an LC_{50} of 176 µg/kg dung fw was derived (Hempel et al. 2006). The PNEC was derived by dividing this LC_{50} by a safety factor of 100 (Liebig et al. 2010).

[g] NOEC values for the abundance of dung fly larvae and dung decomposition were derived in a 86-d field study (Römbke et al. 2010b). Since no guidance on a safety factor to derive a PNEC from such NOEC values is provided by EMEA/CVMP (2008), these NOEC values were directly compared to the refined PEC values (Liebig et al. 2010).

Dung degradation is a complex process that reflects the activity of numerous species in close interaction with abiotic (mainly climatic) and other biotic factors (e.g. birds foraging on pats and vegetation growth). The nutritional value of the dung pats and their physical properties (e.g. moisture content) strongly affect their attractiveness and usefulness for invertebrate species. In fact, the composition of the dung fauna (e.g. the dominance of fly larvae feeding on the dung vs. large beetles removing it from the surface) strongly influences the fate of the dung pats (Hanski & Cambefort 1991). Finally, yet importantly, the properties of the dung itself (including residues of parasiticides) determine how quickly the dung pats are degraded. In temperate regions, the whole process may vary from weeks to years. A recent example, comparing the effects of ivermectin in different ecological regions (Canada, The Netherlands, France and Switzerland) on dung organism diversity and functions, has been provided by Floate et al. (2016) and Tixier et al. (2016). In this context, it should not be forgotten how many different factors are influencing dung decomposition (Figure 22).

Figure 22: Factors influencing the degradation of cattle dung



Presentation based on Floate (pers. comm.), modified.

Finally, the question has been raised whether there really is a problem regarding the diversity of dung organisms. Actually, no detailed investigations of the structure and functions of dung organism communities are available. However, Rössner (2012) showed that 20% and 25%, respectively, of dung-related beetle species are missing or endangered in various biotope types of former East Germany (including protected areas). In the German Democratic Republic (GDR), cattle were mostly kept in stables. Thus, dung beetle populations may have had less access to food. This fact may have contributed to the decrease in diversity and abundance of dung beetles on meadows of the GDR (Rössner 2012). A similar trend has been observed in the former GDR for dung beetles on sheep pastures after 1990. Comparable studies for other parts of Germany are rare, but Reichholf (2007) found a similar decrease in Lower Bavaria between 1969 and 1996 that was attributed to an increase in the percentage of stabled animals. In addition, Hannig & Kerkering (2015) discuss the drastic decrease in numbers of the horned dung beetle (*Copris lunaris*) in Germany, in particular in North-Rhine Westphalia. As reasons for this observation, the authors mention the increased percentage of stabled cattle as well as the usage of avermectins. Currently, the German Federal Agency for Nature Conservation (BfN) is preparing a Red List of dung beetles.

Referring to these numbers, it has to be clarified whether RMMs should focus primarily on endangered species (i.e. those typically found in Red Lists). However, with the exception of certain countries or even regions such information is not (yet) available. In this context, it has to be decided, which protection goals – the diversity or the function of the organism communities – are the most important ones. In case the diversity is protected, the functions provided by this community are 'automatically' protected too, but this is not true the other way around (Stork & Eggleton 1992). However, it should not be forgotten that – besides the use of parasiticides and other veterinary pharmaceuticals – other anthropogenic activities (such as the use of pesticides, inorganic fertilisers and land use changes) may also play a role here.

Risk mitigation measures focusing on the protection of the dung organism community are presented in sections 9.4.1.1–9.4.1.3.

9.4.1.1 Strategic treatment of the animal group / herd is only allowed outside the periods of maximal abundance and diversity of dung organisms

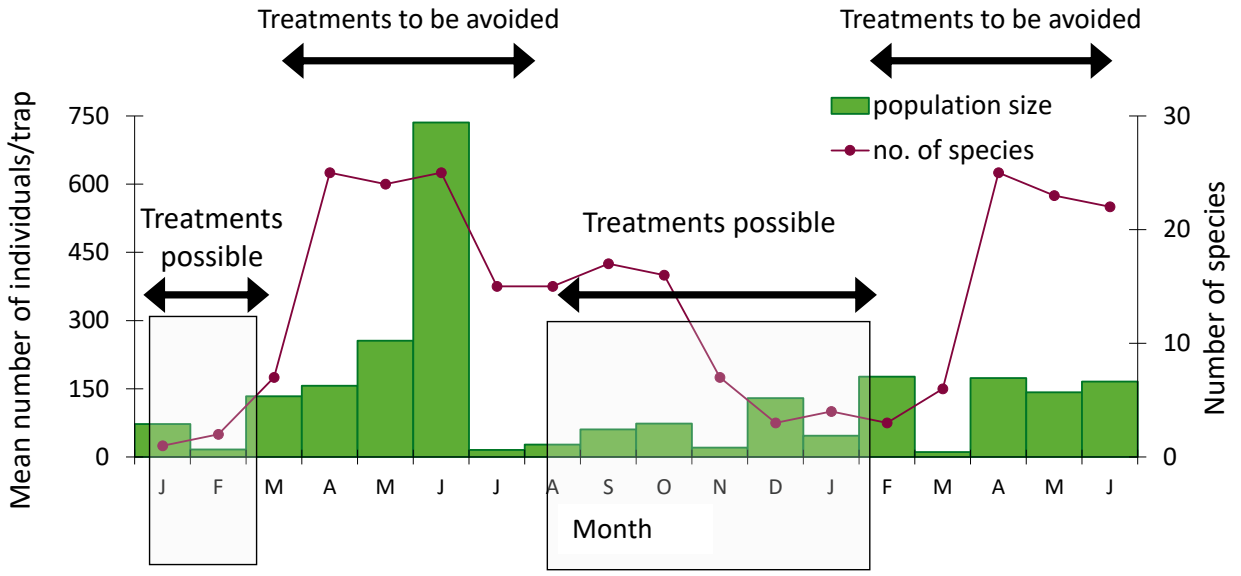
This risk mitigation measure has been included in some SPCs for Flukiver Combi, a combination product containing the antiparasitic agents closantel and mebendazole [21] (see also Liebig et al. 2011). Flukiver Combi is used to treat infestations of sheep with different helminths (e.g. *Fasciola hepatica* and *Haemonchus contortus*) and the sheep bot fly *Oestrus ovis*. Please note that only very general information is provided in these SPCs on the appropriate time of treatment.

Lumaret (2010) observed during his field studies in Southern France that the time and frequency of parasiticide treatments of farm animals and the abundance and biodiversity (number of species) of dung beetles in Southern France follow a specific pattern (Figure 23). He identified certain periods during the year, in which parasiticide treatments do not harm dung beetles since during these time windows the dung beetle species are either not active or not occurring at all (e.g. between September and the end of February/mid of March). The basic idea is that, assuming that certain information regarding the diversity and activity of dung organisms as well as the treatment pattern of livestock is known, it would be possible to use parasiticides without harming the local dung organism community (at least not severely). It should be pointed out that mainly due to climatic factors, both patterns can vary during the course of the year. The same is true for long-term cycles of insect activity patterns, meaning that the relationships between parasiticide treatments and insect activities are not always very clear.

To check the practicability of this RMM, information on the occurrence and reproduction periods of four dung beetle species used regularly in ecotoxicological tests (Lumaret 2010) was compared to the treatment patterns for doramectin, eprinomectin, ivermectin and moxidectin in farms located in southern France (also based on Lumaret 2010; Table 27). Even a quick comparison reveals that there are only few months without treatments and/or without occurrence or reproduction activities of these four beetle species. However, details on the number of treatments that are necessary have not been clarified yet (see section 9.3.1). These details need to be evaluated for each parasiticide, farm animal and parasitosis. In any case, the identification of 'windows of opportunity' seems to be difficult, especially when considering that in reality the number of dung beetle (and, more generally, dung organisms) species will be higher.

[21] Mebendazole and closantel have potentially toxic effects on dung organisms. In order to limit their impact on dung fauna, systematic mass treatments should be administered only in autumn, after the fly season, or in the early spring. In addition, it is recommended that sheep and lambs should not be turned onto pasture within seven days after treatment' (Health Products Regulatory Authority 2014).

Figure 23: Seasonal variation in the abundance and biodiversity (= number of species) of dung beetles in Southern France juxtaposed with possible treatment periods of farm animals



Presentation based on Lumaret (2010) and Adler et al. (2013), modified.

Table 27: Comparison between the treatment patterns for doramectin, eprinomectin, moxidectin and ivermectin and the activity patterns of four dung beetle species. Treatment patterns are presented as percentages of the pasture area, on which cattle was treated (L: 1-20%, M: 20-50%, H: 50-100%). They were obtained in a poll at 300 farms located in southern France (Lumaret 2010). Activity patterns are indicated for four dung beetle species often used in ecotoxicological tests (O: occurrence period, R: reproduction period). The activity patterns, which do not refer to a specific region, are based on Lumaret (2010).

Parasiticide	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Doramectin					L			M		M	M	
Eprinomectin				L			L	L		M	M	
Moxidectin									H			
Ivermectin		L	L	L	L	L	L		L	M	L	
Dung beetle species												
Aphodius constans	R	R	R	O								O
Aphodius porcus								O	R	R	O	
Onthophagus taurus				O	O	R	R	R	O	O		
Onthophagus vacca				O	R	R	O	O	O	O		

Moreover, it has to be pointed out that while the individual farmer knows when he is using which parasiticide in what amount, there is so far no chance to get data on the occurrence and/or activity patterns of dung organism species. This is due to the fact that such data have never been collected or – in case they exist – are not publicly available. In order to underline this point an effort was made to collect ecological data on common European dung beetles as part of a research project supported by the UBA (Römbke et al. 2017). Referring to this data compilation, the following conclusions regarding the availability of data on dung organism distribution and/or activity patterns were drawn:

- For many species, only sporadic information on individual sampling is available. Most samplings just contain the species name, the name of the site, the date of the sampling and the name of the collector. Without data describing the site conditions, the sampling method, the climatic conditions during sampling (to name just the most important), it is impossible to develop distribution maps for individual species.
- Available maps mainly show regional sampling activities but not the actual range of the respective species (see Römbke et al. 2017). In fact, no organised monitoring of dung organisms is known from Europe.
- Observations on the phenology, ecology, life cycle or feeding preferences of dung organisms are often scarce and have not been collected according to a specific sampling protocol or standard method.
- In case data are available, they refer to few charismatic (i.e. large or 'beautiful') species, i.e. mainly beetles such as *Onthophagus taurus* or the yellow dung fly *Scathophaga stercoraria*. Corresponding data on dipteran or nematode species are missing.
- To our knowledge, there are no publicly usable databases on the biodiversity or ecology for at least the most common dung organism species (however, discussions have started to set-up such databases, e.g. in Canada; K. Floate, pers. comm.).
- In order to obtain taxonomic, ecological and biogeographical information for dung organisms large-scale monitoring programs have to be set up: (a) in regions without recent usage of parasiticides (in order to know the normal diversity and abundance of dung organisms) and (b) at sites, where parasiticides have been used. In the latter case, the amount and frequency of the usage of parasiticides must also be known.
- Even in case all required information would be available, it is highly probable that the necessary time windows (i.e. a period when a treatment is necessary and can be performed, since the dung organisms are not active) are not found. In addition, the information has to be made public, preferably in central databases. Such an effort would not only be useful for the risk assessment of parasiticides and other veterinary pharmaceuticals, but also for the general protection of biodiversity.
- Finally, it has to be checked whether the application frequencies as shown in Table 27 are really necessary. A reduced number of treatments might be sufficient to control parasites (and even beneficial in view the development of resistance; see sections 6 and 9.3.1).

In summary, there is no specific season of activity of dung organisms. Different species are active at different times of the year and this pattern depends on the geographic/climatic region. The present knowledge on the biology and ecology of dung organisms is insufficient to identify appropriate time windows, during which parasiticides could be administered without harming dung organism communities, i.e. to sufficiently specify the RMM. Currently, it appears unlikely that time windows, which are appropriate for treating farm animals on the pasture and during which dung organisms are inactive, will be identified.

To evaluate if the RMM is practicable for cattle, sheep and horses, comprehensive data on the usage of parasiticides are needed. This includes information on the time / frequency of application for each parasiticide in the different livestock animal species, breeds and age classes for each farming method / husbandry system (cf. sections 9.3.1 and 9.4). A detailed evaluation is required for each situation. Restrictions of the time, during which a parasiticide can be applied, have to be made for each livestock

species and indication in close cooperation with parasitologists as emphasised at the project workshop (see section Annex 2). In this context, possibilities to optimise the treatment regime should be considered (section 9.3.1). For instance, young farm animals have to be treated when infection pressure is high (e.g. several weeks after the start of the grazing season, depending on the parasite species, see section 9.3.1). Such a treatment is likely to coincide with periods during which dung organisms are active and abundant (Figure 23, Table 27). Yet, during their first grazing season young animals should preferably be kept together with older animals to reduce the infection pressure. In this case, a selective treatment of the young animals could be carried out instead of a strategic treatment (section 9.3.1), so that dung without parasiticides would be available.

As outlined above much information on the diversity, occurrence, ecology, behaviour and sensitivity of almost all dung organisms is missing. It is recommended to identify a central institution to collect this information in a publicly available database. In order to overcome taxonomic bottlenecks the use of modern genomic methods is recommended. Furthermore, currently used treatment frequencies should be critically checked and reduced where possible (cf. section 9.3).

9.4.1.2 The product is toxic to dung organism (flies, beetles). Therefore, do not treat animals on the same pasture in successive seasons to avoid adverse effects on dung fauna and their predators

The aim of this RMM is to protect the dung organism community by avoiding repeated input of dung from treated farm animals on a specific pasture, thus giving the communities time to raise generations without being exposed to the parasiticide. According to EMA/CVMP (2012), the term 'successive seasons' means e.g. spring and summer of the same year.

This RMM has been discussed and evaluated in EMEA/CVMP (2008) and EMA/CVMP (2012), but with different outcomes. In EMEA/CVMP (2008), it was classified as appropriate. However, in EMA/CVMP (2012) it was assessed differently based on the following reasons:

- The RMM may be in conflict with the agricultural practice in the respective region, since not all farmers might have the possibility to rotate pastures.
- For reasons of animal welfare, it might not be possible to adhere to this RMM, if no alternative pasture is available.

In this context, it should also be mentioned that due to other legal requirements, some pasture areas (e.g. low-nutrient meadows and dikes) may require grazing in successive seasons.

In addition, it is pointed out in EMA/CVMP (2012) that an assessment of the suitability of this RMM depends on the availability of data from higher tier (i.e. field) studies, but the respective methods are still under development (see also Adler et al. 2016b, Floate at al. 2016).

In their survey, Liebig et al. (2014) state that no examples for usage of this RMM were identified. Principally, the RMM can be used for cattle, horses and sheep kept at least partly on pastures. In some cases, it may already be agricultural practice, since not all pastures are able to provide food for animals in successive seasons. In addition, the RMM is already practised in sheep husbandries with a frequent change of pastures.

If a dung organism species would benefit from this RMM depends on its life cycle and on the time of the antiparasitic treatment on the respective pasture. In general, all multivoltine species (i.e. species with several generations per year, mainly small fly species) would benefit from the RMM. If univoltine species (i.e. those with one generation per year) would benefit from such a measure, depends on the coincidence of the antiparasitic treatment and the reproductive cycle of the respective dung organism species. Thus, it is difficult to predict to which extent this RMM would be effective to protect the dung organism community. On a regional scale, exposure of dung organisms to dung from treated farm animals would not decrease. To date, no data are available on the long-term development of dung organism communities under such an exposure scenario, lasting over several seasons, or, years.

It should also be mentioned that the use of this RMM might help to reduce the development of resistance among parasite populations and the number of parasites excreted on a specific pasture (see section 6).

A necessary precondition for applying this RMM is that rotational grazing schemes are implemented (EMA/CVMP 2012). If a sufficiently large pasture area relative to the number of grazing animals is available, it should be feasible to implement such grazing rotations.

Such rotation schemes are e.g. used in the German state of North Rhine-Westphalia, where the following systems are relatively common (LWK-NRW 2015):

- Rotational grazing systems with pastures divided in several (about 10) plots, which are subsequently used from early to late summer ('Umtriebsweiden'). Farm animals are grazing on such plots for about three days, before rotating to the next plot. The grazed plots are kept without farm animals for 2–3 weeks in early summer and for 4–6 weeks in late summer.

- Temporary strip-grazing systems ('Portionsweiden') with a daily re-arrangement of grazing plots by using transportable electric fences. Disadvantages of this system are the high amount of work and the usually higher stocking density, which in turn increases the probability of soil compaction.

In addition to the rotation schemes mentioned above, a rotation between treated and untreated farm animals kept on a specific pasture (or grazing on separate plots of this pasture) is possible. Both treated and untreated groups of animals would be present, if deworming activities are not applied to the whole stock but targeted, which is increasingly the case (see section 9.3.1).

In summary, this RMM is suitable to protect multivoltine dung organisms. If univoltine species would benefit from the RMM, depends on the overlap of their reproductive cycle and the time of antiparasitic treatment.

Principally, the RMM appears practicable for cattle, horses and sheep, with its practicability mainly depending on the availability of a sufficiently large pasture area (relative to the number of grazing animals) allowing the implementation of a rotational grazing scheme. If and to which extent the RMM can be implemented in routine farming practices, has to be evaluated for each situation. In the context of the RMM, the required rotation frequency depends on the frequency of application of the parasiticide in the respective animal species, breed, age class and farming / husbandry system. Compliance with the RMM is easy in rotational grazing systems with frequent rotation such as those described above (LWK-NRW 2015) and in sheep husbandries with a frequent change of pastures. It will probably be difficult or impossible in cattle and horse farms with a limited pasture area as was pointed out at the project workshop (see section Annex 2). An important factor is whether farmers in a certain region have the appropriate experience and resources to apply rotational grazing schemes. It should be evaluated how such a measure could be included in routine work plans on the farm level, in order to minimise the additional workload and, thus, increase the acceptance of this RMM. Rotational grazing schemes do not only lead to the protection of dung organisms, but also help to reduce the parasitic infection pressure on the pastures. Therefore, the cost-benefit analysis of such schemes should be favourable, a fact that is likely to help increasing the level of acceptance by farmers.

In this context, it should also be mentioned that if a targeted treatment is carried out, and treated and untreated animals (e.g. young treated animals and older, non-treated animals) graze together on a pasture (see section 9.3.1), both contaminated and uncontaminated dung is present on this pasture. Hence, targeted treatments appear to be an alternative to the evaluated RMM.

When specifying this RMM, other parasiticides with the same or a similar mode of action should also be considered. This means that it should be avoided to treat animals on the same pasture during successive seasons with different active ingredients having the same or a similar mode of action.

9.4.1.3 Animals from free-range husbandry must be stabled during treatment and for X days following treatment

This RMM has especially been included in the SPC for antiparasitic products used for horses, e.g. for Equest Pramox 19.5 mg/g + 121.7 mg/g oral gel, a combination product containing moxidectin and praziquantel [22] (see also Liebig et al. 2011) for the treatment of horses infested e.g. with strongyles, ascarids (*Parascaris equorum*) and tapeworms. It is also mentioned – in combination with the RMM discussed in section 9.4.2 – in some SPCs for Flukiver Combi (see footnotes 21, section 9.4.1.1, and 22, this section). It is of note that in both cases, it is recommended to stable the animals for a relatively short period (3 and 7 days, respectively).

[22] 'In order to limit the impact of moxidectin on dung fauna, and due to insufficient data regarding environmental risk of praziquantel, horses should not be turned out onto pasture within 3 days of treatment' (Zoetis UK Limited 2016).

'[...] it is recommended that sheep and lambs should not be turned onto pasture within seven days after treatment' (Health Products Regulatory Authority 2014).

Again, the aim of this RMM is to protect the dung organism community by reducing their exposure to parasiticides. The dung of the treated animals, which are kept in stables, should obviously not be spread directly (see section 9.4.2.1).

The RMM requires that the treated farm animals are kept in stables for a certain time, which depends on the excretion profile of the specific parasiticide in the respective animal species. As discussed in section 7, the faecal excretion profile is influenced by the route of application (parenteral, topical or oral), the formulation and the dosage of the parasiticide (see e.g. Aksit et al. 2006, Kolar et al. 2006, Lumaret et al. 2006, Gokbulut et al. 2016). Generally, the highest concentrations of parasiticides are excreted during the first few days after treatment (see also section 9.3.1). The concentration of the excreted parasiticide in the dung has to be compared to effect concentrations, which are usually derived in standardised laboratory tests with dung organisms (see section 8). Using a safety factor of 100, the results of these single-species tests are extrapolated to the whole dung organism community (section 9.4.1). The farm animals have to be stabled until the concentration of the parasiticide in dung is low enough to avoid unacceptable effects on dung organisms. If this is feasible depends on the farm animal species and on the farming system (see below). In addition, animal welfare considerations have to be taken into account, as was also stressed at the project workshop (see section Annex 2).

As mentioned above, peak excretion is often restricted to a few days, meaning that stabling the treated farm animals during this period will reduce exposure of dung organisms in the environment considerably. For instance, peak excretion of avermectins and milbemycins orally administered to horses was recorded on day 2.5 after application. Ninety percent of the applied parasiticides were excreted within the first 4 (ivermectin) or 8 days (moxidectin) after application. In cattle, excretion peaks were found on days 2–9 (parenteral application of ivermectin), days 1–3 (topical application of ivermectin), day 3 (parenteral application of doramectin), day 5 (topical application of doramectin), days 2–3 (topical application of eprinomectin), day 3 (parenteral application of moxidectin) and days 3–5 (topical application of moxidectin). Sixty-five percent of the applied ivermectin dose was excreted 7 days after parenteral treatment. In sheep, peak excretion of doramectin was recorded on days 2–5 following parenteral application (see sections 7 and 9.3.1).

In addition to the calculation of 'safe' concentrations of a parasiticide for dung organisms by using the approach mentioned above, it is possible to evaluate the effects of a parasiticide directly in the field. Unfortunately, with the exception of ivermectin, only relatively few datasets from long-term field studies with parasiticides are publicly available as was also pointed out at the project workshop (cf. Annex 2). In these field studies, it has been investigated when and for how long effects of dung from treated cattle can be observed under real field conditions. Referring again to ivermectin, the highest concentrations are found 2–7 days after application. Effects on beetles are rarely found for periods of longer than a few weeks after application, which means that these – in any case few – field studies are usually run for about a month. However, dipteran flies, especially species of the family Sepsidae, could be affected by dung from treated animals up to 56 days after treatment (Floate et al. 2016). Therefore, ivermectin treated cattle would need to be stabled for more than 56 days to reduce the ivermectin residues in dung to levels below concentrations affecting Sepsidae. It is an open question whether this example really represents a worst-case scenario, at least for two reasons:

- It is not clear whether sepsid species could be affected even longer, since the respective Canadian field test (Floate et al. 2016) was terminated after eight weeks.
- It is not known if these flies are always the most sensitive group.

For a comprehensive assessment, additional factors have to be considered. For example, do these flies have alternatives that are within reach, i.e. dung from untreated cattle kept on the same pasture, if a selective treatment or a targeted selective treatment is carried out (section 9.3.1), or on an adjacent pasture? If yes, is that dung sufficient for keeping the diversity and abundance of the fly population within their normal (i.e. the control) range? If not, how long would it take to get back to this normal

range? In this context, it should also be noted that when the animals are stabled, their dung is not available to dung organisms, a fact that may also influence their abundance (see section 9.4.1).

Table 19 illustrates how different the sensitivity of dung organisms (in terms of effect strength and duration) can be after similar application of the same parasiticide (ivermectin) to cattle at three sites (Römbke et al. 2017). Among beetles, no consistent sensitivity towards the excreted concentrations of ivermectin was recorded. It is not clear whether the different composition of species (although from the same family) or the different ecological conditions at the study sites are responsible for this observation. The low sensitivity of dung and rove beetles at the French site was probably caused by dry weather conditions during the study period (at this site, beetle activity and abundance were lower than average).

Among the flies, some families seem to be less sensitive towards ivermectin than others. For instance, Cecymyidae were almost never affected, while the same ivermectin concentrations always caused large and significant effects on Sepsidae, a family known from laboratory tests as being extremely sensitive towards parasiticides (Blanckenhorn et al. 2013a, b). Based on the limited information available for the Sphaeridae, this family seems to be almost as sensitive as the Sepsidae. Information is patchy, but members of these families have probably more than four generations per year in temperature regions. Yet, these numbers vary strongly, depending on environmental factors such as temperature (Pont & Meier 2002). Larger fly species such as *S. stercoraria* (Scathophagidae) produce less generations, e.g. two to three per year in lowland Switzerland (Blanckenhorn et al. 2010). These multivoltine life cycles may favour recovery of dung flies.

In environmental risk assessment of parasiticides, differences in sensitivity between the few species used in standardised laboratory tests and the multitude of species occurring in the field are covered by safety factors. As mentioned above, a safety factor of 100 is used to extrapolate the results of these single-species tests to the whole dung organism community. However, this safety factor might not be sufficient to account for the interspecies differences in sensitivity. Blanckenhorn et al. (2013a) investigated the sensitivity of various sepsid fly species in laboratory tests with ivermectin. They found that some of these fly species reacted by factors of > 100 more sensitive than the standard test organism *S. stercoraria*.

Table 28: Examples of the effects of several concentrations of ivermectin (mg/kg dw) in cattle dung on different dung organism groups at sites in three different European countries (Römbke et al. 2017). S: small effect; M: medium effect; L: large effect on the abundance and/or diversity of these organism groups compared to the control (dung from untreated cattle), n.a.: not applicable (not determined or lacking).

Field site	Montpellier (France)				Zurich (Switzerland)				Wageningen (The Netherlands)			
Time after application [d]	28 d	14 d	7 d	3 d	28 d	14 d	7 d	3 d	28 d	14 d	7 d	3 d
Concentration in dung [mg/kg dw]	0.05	0.69	2.48	2.84	0.05	0.69	2.48	2.84	0.05	0.69	2.48	2.84
Dung beetles												
Hydrophilidae	n.a.	n.a.	n.a.	n.a.	S	S	L	L	S	L	L	L
Aphodiidae	S	S	S	L	S	S	S	S	L	L	L	L
Staphylinidae	S	M	M	L	L	L	L	L	n.a.	n.a.	n.a.	n.a.
Dung flies												
Cecymyidae	n.a.	n.a.	n.a.	n.a.	S	S	S	L	S	S	S	S
Chironomidae	n.a.	n.a.	n.a.	n.a.	S	S	L	L	S	S	S	S
Sepsidae	L	L	L	L	L	L	L	L	L	L	L	L
Sphaeridae	S	L	L	L	n.a.	n.a.	n.a.	n.a.	L	L	L	L

The compiled information shows that right now, it is difficult to identify the duration of the stabling time required to protect the most sensitive dung organisms, because there are not enough effect data to do this in a robust way. Moreover, further information is required on the life-cycle characteristics, the recovery potential and the dispersal behaviour of dung organisms. The application of a safety factor of 100 for extrapolation from single-species laboratory tests to the whole dung organism community is intended to cover rarely studied but (at least in terms of diversity) important groups of dung organisms, but this factor will probably not always be sufficient (see above; Blanckenhorn et al. 2013a).

Whether this RMM is practicable depends on the following factors: (1) the excretion profile of the respective parasiticide (which in turn depends on the farm animal species and breed, the formulation, the administration route and the dose), (2) the effects of this parasiticide on various dung organisms (see section 8), and (3) the farm animal species and the farming system. The RMM is likely to be practicable for horses if the time, during which the animals have to be stabled, is relatively short (see above). For cattle, the RMM might be practicable, if sufficient stabling facilities are available and the pastures are relatively close to the stables. However, this is often not the case as was also pointed out at the project workshop. For sheep husbandries with a frequent change of pastures, the RMM will in most cases not be feasible. Farmers keeping their animals on pastures during the whole year may not have the necessary stabling facilities. Furthermore, a sufficiently large agricultural area is required for applying the manure generated during the time the animals are stabled (see also section 9.4.2.1).

In summary, this RMM appears feasible in farming systems, where the animals are not kept on pastures all-year-round, if the period during which the animals have to be stabled is not too long and the pastures are relatively close to the stables. Stabling the animals during the period of peak excretion of the parasiticide would reduce exposure of dung organisms in the environment considerably, although this approach would not be sufficient to avoid effects on sensitive dung organisms (especially Sepsidae). To further specify this RMM, more information is needed on the ecology of the most important dung organism groups (e.g. duration of life cycles or their horizontal distribution). Since the excretion

profile of a parasiticide depends on the formulation, administration route, dose, farm animal species and breed, the RMM has to be specified accordingly.

9.4.1.4 Overview of the discussed risk mitigation measures focusing on the protection of the dung organism community

An overview of the three discussed risk mitigation measures aiming at protecting the dung organism community, i.e. their diversity and functions, is given in Table 29.

Table 29. Overview of the discussed risk mitigation measures (RMMs) for the protection of dung organisms including a general assessment of efficacy and practicability. Please note that the RMMs have to be specified before a detailed evaluation according to the criteria listed in Table 25 can be performed for each parasiticide, farm animal species and farming system.

Risk mitigation measure	Efficacy to reduce risk	Practicability	Data gaps, recommendations, remarks
Strategic treatment of the animal group / herd is only allowed outside the periods of maximal abundance and diversity of dung organisms.	**Probably not** It appears unlikely that appropriate time windows, during which dung organisms are inactive, will be identified.	**No** The possibilities to shift treatment times are limited. Especially young animals have to be treated when infection pressure is high.	Information on the diversity, occurrence, ecology, behaviour and sensitivity of most dung organisms is missing. Currently used treatment frequencies should be critically checked and reduced, where possible. Where possible, selective or targeted selective treatments should be used instead of strategic treatments.
The product is toxic to dung organism (flies, beetles). Therefore, do not treat animals on the same pasture in successive seasons to avoid adverse effects on dung fauna and their predators.	**Yes** (for multivoltine species) **Possibly** (for univoltine species) If a dung organism species would benefit from this RMM depends on its life cycle and on the time of the antiparasitic treatment.	**Generally, yes** The practicability depends on the availability of a sufficiently large pasture area allowing the implementation of a rotational grazing scheme.	If and to which extent the RMM can be implemented in routine farming practices, has to be evaluated for each farm animal species and farming system. When specifying this RMM, other parasiticides with the same / a similar mode of action should also be considered. This means that it should be avoided to treat animals on the same pasture during successive seasons with different active ingredients having the same / a similar mode of action. Where possible, selective or targeted selective treatments should be used instead of strategic treatments.
Animals from free-range husbandry must be stabled during treatment and for X days following treatment.	**Possibly** Dung organisms would benefit from this RMM, if the livestock animals can be stabled for a sufficiently long period. However, to protect the most sensitive dung organisms, this period may be impracticably long.	**Possibly** The RMM is feasible for farming systems, where the animals are not kept on pastures all-year-round, if the period during which the animals have to be stabled is not too long and the pastures are relatively close to the stables.	The RMM has to be specified for each parasiticide product, administration route, dose, farm animal species and breed. More information is needed on the ecology of the most important dung organism groups (e.g. duration of life cycles, horizontal distribution) and on their sensitivity towards parasiticides.

9.4.2 Risk mitigation measures focusing on the protection of the soil organism community, i.e. their diversity and functions

Livestock animals are treated with parasiticides while on the pasture or while they are stabled (see section 2). The focus of the present project was mainly on RMMs aiming at protecting the dung organism community, i.e. on RMMs applied to animals on pasture. However, at the third project meeting (April 27th, 2016) it was decided that three RMMs for the protection of soil organisms should be included in the evaluation, also in view of the RMM discussed in section 9.4.1.3 ('Animals from free-range husbandry must be stabled during treatment and for X days following treatment').

The concentrations of parasiticides in soil, to which manure is applied, are obviously lower than the concentrations of parasiticides in the dung of treated farm animals. In addition, the toxicity of many parasiticides to soil organisms (e.g. springtails and in particular earthworms) is lower than their toxicity to dung organisms (especially dung flies). Hence, risk quotients for soil (see examples in Table 31, section 9.4.2.2) are much lower than risk quotients for dung (Table 26, section 9.4.1). Still, laboratory multi-species tests (usually with springtails, enchytraeids and/or predatory mites) indicate that arthropods such as springtails (Collembola) could be at risk at relevant parasiticide concentrations (Jensen et al. 2009).

Risk mitigation measures focusing on the protection of the soil organism community are discussed in sections 9.4.2.1–0.

9.4.2.1 Manure from treated animals must be stored for X months prior to spreading on and incorporating into land to allow for degradation of the active substance prior to release into the environment

This RMM, which is discussed and evaluated in EMA/CVMP (2012) and Liebig et al. (2011), can be applied to manure that is stored in manure storage tanks or dung heaps prior to application to land. This manure is in most cases generated by animals that are stabled throughout the year or by grazing animals that are stabled temporarily (e.g. during winter) and treated during the stabling period. Thus, the measure is relevant for cattle and horses, but in most cases not for sheep. In this context, it is of note that due to a higher risk of infections, animals that are kept partly on pastures and partly in stables are expected to be treated more frequently with parasiticides than animals that are exclusively kept in stables.

The RMM is only efficient, if the parasiticide is degraded to a sufficient extent during manure storage resulting in a predicted environmental concentration in soil (PEC_{soil}), which does not pose any risk to the terrestrial environment taking assumptions such as the fraction of the herd that is treated into account (see below). For each parasiticide, the minimum storage time required to reduce the risk to an acceptable level has to be derived based on (1) its degradation in stored liquid manure or dung of the relevant animal species, (2) the corresponding predicted environmental concentration in soil (PEC_{soil}), and (3) the predicted no effect concentration for soil organisms ($PNEC_{soil}$). The derived minimum storage time should lead to a reduction of the risk quotient (i.e. the ratio of PEC_{soil} to $PNEC_{soil}$) below 1.

In the risk assessment for the soil environment, an initial PEC_{soil} is derived using the total residue approach, which is based on the simplifying worst-case assumption that the total applied dose of the pharmaceutical is excreted by the animal. Further assumptions, e.g. the number of days of treatment, and the fraction of the herd that is treated, are also taken into account (Phase II, Tier A, VICH 2005, EMEA/CVMP 2008 [23]). If the risk quotient is ≥ 1, a risk to the soil compartment cannot be excluded. In this case, a refined PEC_{soil} ($PEC_{soil\ refined}$) is derived taking metabolism, excretion pattern and further degradation processes into account (VICH 2005, EMEA/CVMP 2008). One of these degradation processes is the substance degradation in stored liquid manure or dung.

[23] For an example for a comprehensive environmental risk assessment for ivermectin, see Liebig et al. (2010).

Calculating $PEC_{soil\ refined}$ following degradation in stored liquid manure or dung requires information on (1) common manure storage times, and (2) half-life times (DT_{50} values) of the active substance in liquid manure or dung. In EMEA/CVMP (2008), a default value of 91 days is provided for manure storage time for cattle and horses. Animals can be treated at any time during the period they are stabled. If they are treated towards the end of storage period of the manure or dung, there is less time for the active substance to degrade. Therefore, half of the default manure storage time (i.e. 45.5 days) is used in the PEC_{soil} refinement (see EMEA/CVMP 2008).

With regard to half-life times in liquid manure or dung, data for parasiticides (including ivermectin, doramectin, eprinomectin and moxidectin) are lacking. In a recent review of literature data on the occurrence and transformation of veterinary pharmaceuticals and biocides in manure, 684 citations from the years 2000–2015 were evaluated. None of these citations deals with the degradation of parasiticides (Düring et al. 2016, Wohde et al. 2016).

The fate of ivermectin (Sommer & Steffansen 1993, Herd et al. 1996, Alvinerie et al. 1998, Fernandez et al. 2009), moxidectin (Pérez et al. 2001, Hempel et al. 2006, Suárez et al. 2009) and doramectin (Kolar et al. 2006, Kožuh Eržen et al. 2007) in dung pats of cattle and sheep faeces has been evaluated in a number of studies. These data might be useful as a first indication of the persistence of the parasiticides. However, the results of these studies cannot be extrapolated to conditions in manure storage tanks or dung heaps. Therefore, the data cannot be used to derive minimum storage times for parasiticides in stored liquid manure or dung. Due to the lack of appropriate DT_{50} values for the considered parasiticides in stored manure and dung, a specification of the RMM is not possible (see below).

If such DT_{50} data become available, the risk quotient can be calculated taking degradation in stored manure into account (see EMEA/CVMP 2008). If the risk quotient (i.e. $PEC_{soil\ refined}$ / PNEC) is < 1, half of the default manure storage time (45.5 days, see above) is sufficient to degrade the parasiticide to a level that does not pose a risk to the soil compartment. In this case, no extension of the manure storage time is required. If the derived risk quotient is ≥ 1, the manure storage time, which is needed to reduce the substance concentration to an environmentally safe level, has to be calculated. This could, for example, be achieved by using the same equations as for calculating $PEC_{soil\ refined}$, but setting $PEC_{soil\ refined}$ equal to 0.9 x PNEC. This value is suggested, because it leads to a risk quotient ($PEC_{soil\ refined}$ / $PNEC_{soil}$) below 1. Based on the derived minimum manure storage time, the RMM could be specified for the respective substance, matrix (i.e. liquid manure or dung) and animal species (cattle, horses; see also below).

An increase of the minimum manure storage time requires higher storage capacities for manure. In this context, the recent revision of the German Fertiliser Application Ordinance ('Düngeverordnung', DüV 2017), which aims at effectively limiting nutrient surpluses, should be mentioned. Withdrawal periods for manure application on arable land, grassland and cultivated vegetables have been prolonged. Details are:

- a prolongation of withdrawal periods for liquid manure application to arable land to 4 months (after the last harvest until January, 31),
- a prolongation of withdrawal periods for liquid manure application to grassland to 3 months (from November, 1 to January, 31),
- the possibility of a temporal shift of the withdrawal periods for liquid manure application for up to 4 weeks for arable land and grassland,
- an extension of withdrawal periods for dung (from November, 15 to January, 31), and the possibility of a prolongation of these withdrawal periods by up to 4 weeks.

Due to the increased withdrawal periods, manure storage capacities have to be expanded.

To specify the RMM, half-life times (DT_{50} values) of the active substance in liquid manure or dung of the respective animal species are needed to derive the manure storage time, which is needed to obtain

environmentally safe concentrations of parasiticides (including parasiticides with P or vP properties) during passive storage. Such DT_{50} values should be generated using an internationally agreed test procedure. This comprises the following aspects:

(1) Use of a harmonised test protocol for transformation studies in liquid manure and dung

EMA/CVMP (2011) provides general guidance on how to determine the fate of veterinary pharmaceuticals in manure, which based on OECD test guidelines 307 (OECD 2002a) and 308 (OECD 2002b). However, this guidance is not a technical guideline focusing on methodological details. Such details are addressed in recent reports providing technical guidance for transformation studies for veterinary pharmaceuticals and biocides in liquid manure of cattle and pigs (Hennecke et al. 2015, Herrchen et al. 2016, Junker et al. 2016). This guidance can be used as a basis for evaluating the transformation of parasiticides in liquid manure. Furthermore, it should be possible to adapt the test design for evaluating transformation of parasiticides in stored dung. A harmonized design that yields reliable, comparable results is crucial for generating the DT_{50} data needed to specify the RMM.

(2) Selection of an appropriate test duration for substances with P and vP properties

As the antiparasitics ivermectin, doramectin, eprinomectin and moxidectin are supposed to persist in soil, long half-life times in manure are to be expected. Thus, a standard test duration of at least the abovementioned default manure storage time of 91 days should be selected. To characterise the decline of the test substance concentrations, it might be necessary to further prolong the study, a fact which has to be taken into account before test start, e.g. by including sufficient time points and spare samples. This is a crucial point, since experience (Hennecke et al. 2015 and confidential data of IME) shows that transformation of veterinary medicinal products in liquid manure often follows bi-phasic kinetics.

In this context, it is also relevant, if the parasiticide is mineralised or only transformed to another substance, which might still have effects on organisms in the environment. Especially in a mainly organic material such as manure, the occurrence of non-extractable residues (NERs) has to be checked in order to avoid delayed effects (in case these NERs are re-mobilised).

(3) Need to perform transformation studies in liquid manure and stored dung of each animal species

Since conditions in stored liquid manure substantially differ from conditions in stored dung, DT_{50} values have to be derived for each matrix. Moreover, a transfer of the results of transformation studies between animal species is not feasible. Based on their results obtained with pig and cattle manure, Hennecke et al. (2015) emphasised the need to test liquid manure of each species.

If a practicable prolongation of manure storage periods is not sufficient to obtain an environmentally safe antiparasitic concentration, supportive measures that aim at an enhancement of the transformation rate might be required (Table 30; see also Vidaurre et al. 2016).

Table 30: Potential further measures to enhance the transformation of parasiticides in manure [a]

Measure	Discussion
Enhancement of transformation by increased temperature during passive storage	Increase of temperature enhances degradation. Practicability needs to be discussed (e.g. manure application in summer only)
Enhancement of transformation by composting of dung	Non-extractable residues (NER) might be formed. Evaluation of NER needed.
Enhancement of transformation by periodic changes between aerobic and anaerobic conditions (e.g. by rearrangement of stored dung)	Information on fate of ivermectin, doramectin, eprinomectin and moxidectin under changing aerobic and anaerobic conditions is not available.

[a] A number of other methods aim at reducing the amount of manure or the concentrations of contaminants in the manure (e.g. using the manure for biogas production). However, a discussion of these methods is beyond the scope of the present report).

In summary, the RMM is suitable to protect soil organisms, if the parasiticide is degraded to a sufficient extent during the prolonged period of manure storage. The RMM has to be specified for each parasiticide product considering the DT_{50} of the active substance in stored liquid manure or dung of the respective livestock animal species. However, currently such DT_{50} values are not publicly available. The RMM can be applied, if liquid manure or dung is stored before spreading to land. Hence, it is relevant for cattle and horses, but generally not for sheep. The practicability of the RMM depends on the required storage time for manure of the respective farm animal species containing the parasiticide.

9.4.2.2 When spreading liquid or solid manure from treated animals onto arable land, the maximum nitrogen spreading limit must not exceed X kg N per hectare and year (X < 170)

The European Nitrate Council Directive 91/676/EEC (EC 1991) aims to protect water quality across Europe by preventing the pollution of groundwater and surface waters with nitrates from agricultural sources and by promoting the use of good farming practices. In Germany, this directive is implemented in German law as 'Düngeverordnung' (DüV 2017), which has recently been revised (see section 9.4.2.1). With regard to nitrogen fertilisation management, the most important point is the limitation of the total amount of nitrogen that can be applied to a maximum of 170 kg per hectare and year [24](DüV 2017). Only in exceptional cases, higher amounts can be applied.

The aim of the present RMM is to reduce the introduction of veterinary pharmaceutical products (here: parasiticides) in the soil by further limiting the yearly amount of (liquid or solid) manure applied to a site (Liebig et al. 2011). By doing so, the local exposure of soil organisms is reduced.

The RMM can be applied to (liquid or solid) manure spread to land, i.e. to manure that is in most cases generated by animals that are stabled, either throughout the year or temporarily, and treated during this stabling period. Hence, the measure is relevant for cattle and horses, but generally not for sheep.

Given that the aim of this RMM is to protect the soil organism community, the maximum amount of nitrogen to be applied on crop sites and grassland would need to be determined based on the risk quotient for soil organisms. This risk quotient is the quotient of the predicted exposure concentration (PEC) of the parasiticide in soil and the predicted no effect concentration (PNEC), which is based on the lowest effect concentration determined in laboratory tests with earthworms and springtails (section 9.4.1). These data are available for all authorised parasiticides (with most information being available for ivermectin). If no risk is identified (i.e. the risk quotient is < 1), no risk mitigation meas-

[24] The maximum amount of applied nitrogen depends on the amount of nitrogen removed from the soil by the cultured plants (i.e. with the crop). If less nitrogen is removed, the maximum amount of applied nitrogen is also reduced.

ure is required. In case that a risk is identified (i.e. the risk quotient is ≥ 1), the maximum applied amount of manure (based on the maximum nitrogen spreading limit) would need to be reduced to a value below 170 kg per hectare and year until the risk quotient is < 1.

As an example, PECs for ivermectin in soil after application of manure from intensively reared animals, PNECs for soil organisms and the resulting risk quotients are summarised in Table 31 using data from Liebig et al. (2010). The refined PEC values ($PEC_{soil\ refined}$) were derived based on EMEA/CVMP (2008) considering degradation in manure and soil. Since no data on the degradation of ivermectin in stored manure were available (see also section 9.4.2.1), data for the degradation in soil-faeces mixtures were used instead. Depending on the ivermectin dosage and application frequency, the used DT_{50} values for the soil/faeces mixture and soil, and the manure-spreading scenario (for details see Table 31), $PEC_{soil\ refined}$ ranged from 0.5 to 11.4 µg/kg soil dw. As pointed out by Liebig et al. (2010) the refined PEC values were in some cases higher than the corresponding initial PEC_{soil} values derived according to EMEA/CVMP (2008) using the total residue approach. This fact indicates that ivermectin is likely to accumulate in soil.

The lowest effect concentration for soil invertebrates was determined in a two-species test with the springtail *Folsomia fimetaria* and the predatory mite *Hypoaspis aculeifer*. Based on the EC_{10} of 20 µg/kg soil dw for reproduction of *F. fimetaria* (Jensen et al. 2009) and a safety factor of 10, a PNEC of 2 µg/kg soil dw was derived (Table 31). While no risk is expected at the lowest $PEC_{soil\ refined}$ (risk quotient: 0.3), the highest $PEC_{soil\ refined}$ is expected to result in a risk (risk quotient: 5.7). For all cases where a risk is indicated, the RMM would need to be specified, i.e. a maximum nitrogen spreading limit < 170 kg per hectare and year would need to be derived for each parasiticide product, animal species, dosage and application frequency, considering the manure-spreading scenario.

Table 31: Refined predicted environmental concentrations (PECs) for ivermectin in soil after application of manure from intensively reared animals, predicted no effect concentrations (PNECs) for ivermectin and soil organisms and the resulting phase II tier B risk quotients (data from Liebig et al. 2010).

$PEC_{soil\ refined}$ (µg/kg soil dw) [a, b, c, d]	PNEC (µg/kg soil dw) [e]	Risk quotients
0.5 – 11.4 [e]	2.0	0.3 – 5.7

[a] PECs were derived according to EMEA/CVMP (2008) for various ivermectin-containing products for different livestock animal species and ages (including calf, dairy cow, beef cattle, weaner pig and horse), using the information on dosage (0.1–0.5 mg/kg bw) and application frequencies (1, 2, or 7 applications) provided in the respective summaries of product characteristics (Liebig et al. 2010).

[b] PECs were calculated based on EMEA/CVMP (2008) taking degradation in manure (see below) and soil (DT_{50} values of 16 and 67 d, Krogh et al. 2009) into account. Due to the lack of data on degradation of ivermectin in stored manure, data for the degradation in soil-faeces mixtures (DT_{50} values ranging from 7 to 240 d, Halley et al. 1989a, Boxall et al. 2002) were used instead. However, degradation in (mainly aerobic) soil-faeces mixtures may differ significantly from degradation in stored manure, where anaerobic degradation is most relevant (Liebig et al. 2010).

[c] The default values provided by EMEA/CVMP (2008) were used for manure storage time and nitrogen produced during storage. As specified in EMEA/CVMP (2008), it is assumed that the maximum amount of nitrogen (170 kg per hectare and year) is applied.

[d] The highest PEC values were derived using the worst-cases assumptions of $DT_{50\ soil/faeces}$ = 240 d and $DT_{50\ soil}$ = 67 d assuming a scenario of 5 manure spreading events on grassland with 2-months intervals.

[e] In a 21-day test with two soil invertebrate species, the springtail *Folsomia fimetaria* and the predatory mite *Hypoaspis aculeifer*, an EC_{10} of 20 µg/kg soil dw was derived for reproduction of *F. fimetaria* (Jensen et al. 2009). The PNEC was derived by dividing this EC_{10} by a safety factor of 10 (Liebig et al. 2010).

Test guidelines are available for determining effects on soil invertebrates (OECD 2016a-c) and degradation in soil (OECD 2002a), and the results of such tests are included in product dossiers submitted for parasiticides. By contrast, there is so far no test guideline for evaluating the degradation of parasiticides in stored manure, although some guidance has recently been developed (cf. section 9.4.2.1). Moreover, publicly available DT_{50} values for avermectins and milbemycins in stored manure are lacking (see above and section 9.4.2.1). Yet, data on degradation in stored manure are required to derive reliable PEC soil refined values. In the absence of DT_{50} values for manure, initial PEC_{soil} values or, for persistent compounds, $PEC_{soil\ plateau}$ values (PEC values after application in subsequent years; EMEA/CVMP 2008) would need to be used to derive the risk quotients, which are the basis for deciding if and to which extent the maximum applied amount of manure would need to be reduced to a value < 170 kg per hectare and year.

Generally, if this RMM is implemented, sufficiently large agricultural areas have to be available that can be used for application of the manure. In regions, where many farm animals are kept (e.g. Lower Saxony in Northern Germany) and where certain soil characteristics prevail (light sandy soils, in which nitrogen is easily leaching into the groundwater), this RMM is most likely not practicable, since the agricultural areas, on which the manure can be applied, are limited. Alternatively, the manure has e.g. to be sold to other regions, where its use as a fertiliser is possible or it could be used for biogas production (see Vidaurre et al. 2016).

In EMA/CVMP (2012), all RMMs that may involve sale of manure are critically addressed, since the farmer who is going to spread the manure might not be informed about this RMM. It is concluded that such mitigation measures may only be suitable for countries without manure trading or prior authorisation of disposal of manure. However, the spreading of manure on land usually is a highly regulated process. Any farmer producing and applying manure should be informed about his duties, including the necessity to know how much manure (i.e. nitrogen) is allowed to be applied. The fact that information on a reduced maximal amount of nitrogen to be applied per hectare and year has to be passed on from the farmer selling the manure to the manure trader and, finally, to the farmer applying the manure should not limit the practicability of this RMM. However, measures would need to be implemented to ensure that relevant information is passed on from the farmer producing the manure to the farmer applying the manure. In this context, it should be pointed out that RMMs can generally only be effective, if all relevant information is communicated (e.g. from the veterinarian to the farmer) and put into practice (see also Vidaurre et al. 2016).

In summary, this RMM is suitable to protect soil organisms. The reduced maximal amount of nitrogen to be applied per hectare and year needs to be specified for each parasiticide product, animal species, dosage, application frequency and manure-spreading scenario, in case that the respective risk quotient is ≥ 1. The risk quotient should preferably be based on refined PEC_{soil} values. However, DT_{50} values of the parasiticide in stored manure of the relevant animal species are needed to derive $PEC_{soil\ refined}$. In the absence of such DT_{50} values, $PEC_{soil\ initial}$ or, for persistent compounds, $PEC_{soil\ plateau}$ may need to be used instead. The RMM can be applied to manure spread to land and is, thus, relevant for cattle and horses, but in most cases not for sheep. When the RMM is implemented in regions where farm animals are intensively kept (e.g. north-western Germany), it might be difficult to find enough sites where the manure could be spread. If the manure is sold, it has to be ascertained that information on the reduced maximal amount of nitrogen to be applied per hectare and year is passed on from the farmer selling the manure to the farmer applying the manure.

9.4.2.3 Manure containing the active substance should not be spread on the same area of land in successive years to avoid accumulation of the active substance, which may cause adverse effects on the environment

As the previously mentioned RMM (section 9.4.2.2) this measure, which is mentioned in EMA/CVMP (2012), aims to reduce the introduction of parasiticides in the soil by limiting the amount of manure applied per site and time. In particular, the accumulation of persistent parasiticides in soil should be avoided. By doing so, the exposure of and, thus, the effects on soil organisms are reduced. While the previous RMM focused on the limitation of the input of manure by further limiting the amount of applied nitrogen, this RMM aims at preventing the accumulation of the respective parasiticide in soil by reducing the number of successive manure applications.

As the previous two measures (sections 9.4.2.1 and 9.4.2.2), this RMM can be applied to manure spread to land, which is generally produced by animals that are stabled, either throughout the year or temporarily. The measure is relevant for cattle and horses, but of little relevance for sheep.

The first question is whether the parasiticide can be expected to accumulate in soil. This information should be available, since soil degradation data from laboratory tests (OECD 2002a) are required during the authorisation process. The RMM could for instance be requested for substances that have (1) a $DT_{50\ soil}$ > 120 days (i.e. are persistent in soil; EC 2011) or (2) a $DT_{90\ soil}$ > 1 year. According to VICH (2005) and EMEA/CVMP (2008), substances with a $DT_{90\ soil}$ > 1 year are likely to accumulate in soil and, therefore, $PEC_{soil\ plateau}$ has to be derived. Alternatively, $PEC_{soil\ plateau}$ could be derived and compared with the PNEC for soil organisms to assess whether this accumulation could lead to a possible risk. Probably, effect concentrations for springtails, which are often reacting more sensitive to veterinary medicinal products (VMPs) than earthworms (Jensen et al. 2003, 2009, Liebig et al. 2010), are most relevant (see also example in section 9.4.2.2). A consistent approach should be used to decide if this RMM should be implemented.

To find out if such an accumulation is indeed occurring in soil and if it could have adverse effects on soil organisms, the actual concentrations of the respective parasiticide in agricultural soil, to which manure containing the parasiticide has been applied during successive years, would need to be determined. However, so far such measurements are neither part of field tests with VMPs (note that so far, no standardised method is available) nor of monitoring studies at pasture sites where records of VMP applications are available. Targeted environmental monitoring studies (i.e. long-term field studies under farm conditions) could be used evaluate the potential accumulation of parasiticides applied during successive years and effects on dung and soil organisms (Römbke & Duis 2018).

As discussed for the previous RMM, sufficiently large agricultural areas have to be available to apply the manure. In regions where large numbers of farm animals are kept, this can be difficult, so that the manure would need to be sold (an issue that is critically addressed in EMA/CVMP 2012; see section 9.4.2.2). Alternatively, it could be used for biogas production (see Vidaurre et al. 2016).

In this context, it is interesting that for a similar risk mitigation measure [25] EMA/CVMP (2012) requires that the SPC should include information that a similar risk may exist if other VMPs containing the same (or related) active substance(s) are applied at the same site. If this is the case, the concentrations of all such VMPs would have to be assessed together. However, no recommendations are given by EMA/CVMP (2012) how this could be done in practice.

To summarise, the present RMM is principally appropriate to reduce the accumulation of a parasiticide in soil and, thus, to protect soil organisms. A consistent approach based on a $DT_{50\ soil}$ > 120 days, a $DT_{90\ soil}$ > 1 year and / or a $PEC_{soil\ plateau}$ / PNEC ratio ≥ 1 should be used to decide whether this RMM should

[25] The product can only be used in the same production cycle for X treatment period(s) to avoid accumulation of the active substance in soil resulting in a risk for the terrestrial environment and a contamination of groundwater with the active substance (cf. EMA/CVMP 2012).

be implemented. The RMM can be applied to manure spread to land and is, therefore, relevant for cattle and horses, but typically not for sheep. In regions with intensive animal farming, sufficiently large agricultural areas to apply the manure might not be available. When the manure is sold, information on the parasiticide used to treat the animals, which have produced the manure, has to be passed on from the farmer selling the manure to the farmer applying the manure.

9.4.2.4 Overview of the discussed risk mitigation measures focusing on the protection of the soil organism community

An overview of the three discussed risk mitigation measures aiming at protecting the soil organism community is given in Table 32.

Table 32. Overview of the discussed risk mitigation measures (RMMs) for the protection of soil organisms including a general assessment of efficacy and practicability. Please note that the RMMs have to be specified before a detailed evaluation according to the criteria listed in Table 25 can be performed for each parasiticide, farm animal species and farming system.

Risk mitigation measure	Efficacy to reduce risk	Practicability	Data gaps, recommendations, remarks
Manure from treated animals must be stored for X months prior to spreading on and incorporating into land to allow for degradation of the active substance prior to release into the environment.	**Possibly** Soil organisms would benefit from this RMM, if the manure can be stored long enough, so that the parasiticide is degraded to a sufficient extent.	**Possibly** Whether the RMM is practicable, depends on the required storage time for manure of the respective farm animal species containing the parasiticide.	The RMM has to be specified for each parasiticide based on its DT_{50} in stored liquid manure or dung of the respective farm animal species. At present, such DT_{50} values are not publicly available.
When spreading liquid or solid manure from treated animals onto arable land, the maximum nitrogen spreading limit must not exceed X kg N per hectare and year (X < 170).	**Yes**	**Possibly** The RMM is practicable, if sufficiently large agricultural areas are available that can be used for application of the manure.	The RMM needs to be specified for each parasiticide product, animal species, dosage, application frequency and manure-spreading scenario, if the risk quotient is ≥ 1. When specifying this RMM, other parasiticides with the same / a similar mode of action should also be considered. If manure is sold, it has to be ascertained that information on the reduced maximal amount of nitrogen to be applied per hectare and year is passed on from the farmer selling the manure to the farmer applying the manure.
Manure containing the active substance should not be spread on the same area of land in successive years to avoid accumulation of the active substance, which may cause adverse effects on the environment.	**Yes**	**Possibly** The RMM is practicable, if sufficiently large agricultural areas are available that can be used for application of the manure.	A consistent approach based on a $DT_{50\ soil}$ > 120 d, a $DT_{90\ soil}$ > 1 year and / or a $PEC_{soil\ plateau}$ / PNEC ratio ≥ 1 should be used to decide whether the RMM should be implemented. When specifying this RMM, other parasiticides with the same / a similar mode of action should also be considered. This means that it should be avoided to spread manure containing different active ingredients having the same / a similar mode of action on the same area of land in successive years. If manure is sold, it has to be ascertained that information on the parasiticide used to treat the animals that have produced the manure, is passed on from the farmer selling the manure to the farmer applying the manure.

9.4.3 Possibilities to restrict the use of authorised parasiticides

In the context of the present project, the aim of a restriction of the use of a parasiticide would be to improve the protection of non-target organisms (here: dung and soil organisms). If the use of a parasiticide is restricted, alternative parasiticides or other measures have to be available to prevent and treat parasitic diseases. The development of new active ingredients having a lower toxicity to non-target animals is a very difficult and, in any case, long-term task, although recent developments in genomic and screening technologies have significantly enhanced the opportunities for target-based identification of novel therapies (Woods et al. 2007). However, there is still a lack of basic knowledge on physiology and ecology of many parasites, which strongly hampers all initiatives to develop new parasiticides (Geary et al. 2015).

With regard to restrictions of use, the following two options would theoretically be possible.

(1) Exchange of currently used active ingredients with other substances, which are similarly efficient against the target organisms but have less unintended effects on dung and soil organisms

The search for parasiticides with lower toxicity to non-target organisms, but similar efficiency against target organisms and high practicability (e.g. easy application) has started, when the effects of avermectins on the environment became obvious (Anderson et al. 1984). Since ivermectin, doramectin, eprinomectin and moxidectin have the same (or a very similar) mode of action, the exchange of one of these parasiticides against another (e.g. using doramectin instead of ivermectin) does not help much. According to our current knowledge, the efficiency of these parasiticides is more or less comparable. Their effects on non-target organisms are sometimes similar, sometimes not – depending on various factors including the site- or region-specific composition of the dung or soil organism communities. Suárez et al. (2003), working in Argentinian grassland, reported that the toxicities of doramectin and ivermectin are similar. According to Floate (2006, 2007), who summarised experiences in various Canadian field studies, the toxicity of these four parasiticides can be classified as follows: doramectin > ivermectin ≈ eprinomectin > moxidectin. Thus, moxidectin appears to have a lower toxicity than the three avermectins (see also section 8)[26]. Yet, it is the only one of the four parasiticides that is bioaccumulative (EMA/CVMP 2016a) and, thus fulfils the PBT criteria according to EC (2011; see Table 33).

Table 33: Overview of the PBT properties of ivermectin, doramectin, eprinomectin and moxidectin according to EC (2011) (based on Adler et al. 2016a).

	Fulfilment of the criteria for		
	Persistence	Bioaccumulation	Toxicity
Ivermectin	yes [a]	no [b]	yes [a]
Doramectin	?	no [b]	yes [c]
Eprinomectin	yes [d]	no [d]	yes [d]
Moxidectin	yes [e]	yes [e]	yes [e]

[a] Liebig et al. (2010)

[b] This project (see sections 4.3 and 4.4)

[c] US FDA (2002)

[d] http://ec.europa.eu/health/documents/community-register/2014/20140321127955/anx_127955_en.pdf

[e] EMA/CVMP 2016a

[26] In this context, it should also be mentioned that moxidectin is less affected by anthelmintic resistances than the avermectins (cf. section 6).

For several active ingredients, in particular avermectins, many data on their fate and effects in the environment are available (see e.g. Lumaret et al. 2012). These datasets include detailed information on effects on dung and, much less, soil organisms (cf. section 8). However, much of this knowledge is not publicly available. Making this information publicly available (preferably in a more detailed way that in a normal publication, i.e. at least including detailed supplementary information) would facilitate a comparative evaluation of the toxicities of the different parasiticides and of possible environmental risks.

Parasiticides not belonging to the avermectins or milbemycins have been studied far less. Some of them, such as the benzimidazoles oxfendazole and fenbendazole have only rarely been tested, i.e. no robust assessment of their risk to dung organism communities can be made (Lumaret & Errouissi 2002, Boxall et al. 2006). Others, such as dicyclanil, which belongs to the pyrimidinamines, are as toxic as ivermectin, at least to dung beetles (Hempel et al. 2006). Pyrethroids, e.g. permethrin and cypermethrin used for topical application to farm animals (as pour-on or as ear tags), are regarded as substances with a high potential toxicity to bees, other beneficial insects and aquatic organisms (e.g. Sattelberger 1999). A pour-on treatment with a pyrethroid may lead to a week-long toxicity to dung beetles (Krüger et al. 1999). Similar observations have been made in Brazil (e.g. Bianchin et al. 1998). Wardhaugh (2006) reviewed the insecticidal activity of synthetic pyrethroids (e.g. cypermethrin, deltamethrin and cyhalothrin) in Australian pasture farming and stated that these compounds can be highly toxic to various species of dung beetles and flies for at least 14 days after treatment. Within an EMA referral procedure, potential PBT properties of deltamethrin were discussed controversially (EMA/CVMP 2013, Ibrahim et al. 2013). Interestingly, Palmquist et al. (2012) mentioned that the agricultural use of pyrethroids is less relevant for their occurrence in the environment than their non-agricultural usages.

Overall, the number of classes of parasiticides is small (see section 5). Most of the currently used parasiticides belong to drug classes, which are known for decades. According to unpublished information, this situation is not likely to change within the near future. The target organisms of these parasiticides are members of the same organism groups as the main non-targets organisms (i.e. arthropods). This is most obvious in the case of flies, where species from both target and non-target organisms can be found in the same genus. Therefore, it is an extremely difficult task to develop parasiticides, which are similarly efficient against the target organisms but less toxic to dung and soil organisms.

(2) Limitations of the applied amount of parasiticides, the application frequency or the way how an already authorised parasiticide is applied (i.e. the overall treatment strategy) may also limit their effects on dung or soil organisms

Generally, it is assumed that these options have already been verified during the development of a veterinary medicinal product (i.e. before a new parasiticide is marketed), since these issues directly influence economic questions (i.e. the costs of an application). However, there are several options to improve the treatment regime as discussed in section 9.3.1. The prudent use of antiparasitics is one of the most promising approaches to reduce negative effects on dung and soil organisms. A central point is to minimise the use of parasiticides by replacing strategic treatments by selective treatment approaches where feasible.

10 General discussion

In this section, the project results are summarised and discussed, considering the discussion at the project workshop on 'Risk management strategies for parasiticides used in pasture animals' held at the Federal Environment Agency (Dessau, Germany) on 18-19 January 2017. At this workshop, results described in sections 3, 4, 7, 8 and 9 of the present report were presented and discussed. The 44 workshop participants represented competent authorities, academia and industry, veterinarians and farmers. For a more detailed protocol of the workshop and a list of workshop participants see Annex 2 of this report. The workshop discussion mainly focused on risk management.

According to the general project structure, the present section is structured along three topics. The results of the laboratory tests on octanol/water partitioning and bioconcentration and the literature studies on excretion data and effects on dung organisms are summarised and discussed in the first three subsections. This is followed by a discussion whether available risk management strategies including sustainable approaches to control parasites and risk management measures (RMMs) are suitable for reducing environmental risks caused by avermectins and milbemycins. Overarching issues and general conclusions are compiled in the last subsection (10.4), while knowledge gaps are addressed in section 11.

10.1 Laboratory tests: octanol/water partitioning and bioconcentration

In order to close data gaps and, thus, to contribute to an overview of the environmental relevance of selected parasiticides regarding their potential to bioaccumulate, octanol/water partitioning coefficients were determined for ivermectin and selamectin, while bioconcentration in fish was investigated for ivermectin and doramectin. Using the slow stirring method (OECD TG 123, OECD 2006), log P_{OW} values of 5.6 and 6.0 were determined for ivermectin and selamectin, respectively. A comparison of the derived log P_{OW} of 5.6 for ivermectin with the previously published and much cited log P_{OW} of 3.2 (Halley et al. 1989c, US FDA 1990) suggests that the latter value underestimates octanol/water partitioning of ivermectin [27]. This may be related to methodological drawbacks when using the shake-flask method (see section 3). This result supports the recommendation that OECD TG 123 should be used for highly lipophilic compounds such as ivermectin and selamectin. The previous lack of robust data for ivermectin, a very well-studied and often used parasiticide, illustrates clearly how much these parasiticides were neglected in the past.

In bioconcentration studies with zebrafish according to OECD TG 305, BCF values of 63–111 for ivermectin and 70–71 for doramectin (related to total radioactive residues and normalised to a 5% lipid content) were determined. These BCFs are much lower than initial worst-case estimates derived from log K_{OW} values of 5.6 for ivermectin (see above) and 4.4 for doramectin (US FDA 2002) using the equation indicated in EMEA/CVMP (2008; see section 4.5). They are clearly below the threshold value of 2000 for the B-criterion specified in Annex XIII of the REACH regulation (EC 2011). As discussed in section 4.5, the derived bioconcentration factors are similar to BCFs determined for avermectin B_1 in different fish species (Wislocki et al. 1989, Van den Heuvel et al. 1996, Shen et al. 2005), but much lower than the recently determined BCF > 2000 for moxidectin (EMA/CVMP 2016a). In view of strong interactions of the three avermectins, but not moxidectin, with the transmembrane transporter P-glycoprotein, active efflux of the avermectins is assumed to be the main reason for their low bioconcentration in fish.

Since avermectins strongly sorb to organic substances, uptake with the food can be expected in environmental organisms (e.g. fish). As discussed at the project workshop, it is not clear whether the derived BCFs allow estimating accumulation after dietary uptake, i.e. biomagnification factors (BMFs). Possible BCF / BMF conversion procedures are currently discussed, e.g. at OECD level (OECD 2017).

[27] In the literature, no log P_{OW} values for selamectin were found.

Available studies on dietary uptake of ivermectin focused on evaluating elimination and, hence withdrawal periods (see section 4.1).

10.2 Literature studies: excretion by pasture animals and effects on dung organisms

Excretion by pasture animals

Data on the excretion of parasiticides are relevant when evaluating sustainable approaches to control parasites and risk mitigation measures. Therefore, publicly available data on the excretion of four avermectins (ivermectin, doramectin, avermectin B_1, eprinomectin) and milbemycins (moxidectin) by pasture animals (cattle, sheep and horses) were evaluated. While the avermectins are only marginally metabolized, moxidectin is metabolized to a larger extent. Both avermectins and milbemycins are mainly excreted via the faeces. As was also stressed at the project workshop, excretion rates depend on the animal species, breed and age, as well as the route of administration, formulation and dosage of the parasiticide. Yet, the publicly available data are too limited to systematically evaluate this variability. Generally, about 90% of the applied dose are excreted within approx. 4–10 days after application, but the parasiticides can be detected for much longer periods in the faeces of the treated animals (see section 7). Overall, the identified excretion data are relatively heterogeneous and only limited data are available for some of the parasiticides.

Effects on dung organisms

Based on the evaluation of literature data, the following conclusions can be drawn, taking into account that only for ivermectin a robust data set (including field results) is available. Doramectin is most toxic to dung organisms, followed by ivermectin and eprinomectin that have a similar toxicity. Moxidectin is the least toxic of these four compounds. Generally, dung flies are reacting more sensitively than dung beetles. Where available, LC_{50} or EC_{50} values of ivermectin, avermectin B_1, doramectin, eprinomectin and moxidectin to dung flies are below 10 µg/kg fw and effect durations range from several days to several weeks (see section 8). For doramectin, eprinomectin, avermectin B_1, and especially emamectin, selamectin and milbemycinoxim, information on the toxicity to dung organisms is very scarce or non-existing.

At the project workshop, the relevance of the presented data on the effects of avermectins and milbemycins on dung organisms was discussed controversially. While a reduced degradation rate of dung can clearly affect the usage of the pastures, the correlation between the toxicity of parasiticides to dung organisms and dung degradation rates has so far not been sufficiently explored. Several studies have clearly shown that without dung organism activities (e.g. feeding on and burying of the dung) degradation is strongly delayed. However, this is not always the case: in some field studies no significant effects on dung degradation were found. Differences between field studies are likely to be caused by factors such as the time of the antiparasitic treatment and the species composition of the dung organism community (see sections 8.1 and 9.4.2.1). However, effects on the biodiversity of dung organism groups (especially on dung flies) were found in all field studies with parasiticides. In this context, the question was raised how quickly dung is recolonised. Such a recolonization is restricted by the fact that many dung-inhabiting species have a limited 'window of opportunity' depending for example on the consistency of the dung (see e.g. Lumaret 2010). In this context, it was noted that livestock animals in a certain region are rarely treated simultaneously. Due to this fact, dung without parasiticides should often be available as alternative food source for dung organisms. If and to which extent this is indeed the case remains to be evaluated. Again, this question could be addressed in standardised field studies. The draft guideline published by EMA/CVMP (2016b) could be adapted accordingly.

10.3 Risk management

Sustainable approaches to control parasites

In view of animal welfare and the epidemiology of relevant parasites in the different livestock animal species, parasiticides are a central component of strategies to control parasites as was also stressed at the project workshop. A prudent usage of parasiticides was identified as key factor to reduce effects on the environment: within integrated treatment programmes including complementary prophylactic measures, the frequencies of parasiticide treatments should be reduced to the minimum required to sufficiently control parasitoses (section 9.3.1). In addition to reducing effects on non-target organisms, such an approach would help to prevent the further development of anthelmintic resistances (cf. sections 6 and 9.3.1). Strategically useful times of treatment have to be chosen. As also emphasised during the workshop, animals have to be treated when infection pressure is high. For this reason, the possibilities to shift treatment times in order to reduce effects on the environment (e.g. by treating animals before being turned out to the pasture) are limited.

Generally, the success of antiparasitic treatments should be evaluated regularly. At the workshop, it was also suggested that in view of possible resistances, the respective competent authorities should be informed about lacking treatment efficacies. Data on treatment efficacies and resistances should be collected and evaluated by a central institution. In this context, it was noted that so far no standardised methods for evaluating resistances to parasiticides are available.

Where possible, selective treatments or targeted selective treatments should be used instead of strategic treatments, i.e. only a part of the herd should be treated. If this approach is applied, lower amounts of parasiticides are used, and refugia are available for susceptible parasites (see also below) and dung organisms (see section 9.4.1). Workshop participants pointed out that targeted selective treatment procedures are often known and that they are used in a part of the farms. However, the feasibility of such treatment approaches depends on the possibilities to identify the animals that have to be treated, and to select the optimal time for diagnosis and therapy. In this field, there is still a need for research. It should also be kept in mind that non-immune young animals, which are for the first time on the pasture, have to be treated.

Refugia, in which susceptible parasites survive, should be preserved to prevent the further development and distribution of parasiticide resistances. In addition, a low infection pressure on the pasture leads to the development of a protective immunity within the livestock animals.

Overall, the situation is very diverse involving different livestock animal species, breeds and age classes, various farming / husbandry systems, different parasites differing in their developmental cycles, various epidemiological situations, as well as several parasiticides and application forms. As was highlighted at the project workshop, case-specific approaches are required to effectively and sustainably control parasites. In order to develop such approaches, monitoring data on the prevalence of parasites in farms, the usage of parasiticides, the success of antiparasitic treatments, and the resistance situation in parasites would be extremely useful (see also section 11). Right now, such information is not available.

Further research is required with regard to possible alternative measures to control parasitoses as, for example, vaccination and condensed tannins (section 9.3.4). Existing breeding programs for resistant pasture animals might become more important in the future, but require governmental funding as was also stressed at the project workshop.

Risk mitigation measures

In the following, the evaluated risk mitigation measures (RMMs) for the protection of dung and soil organisms are briefly discussed with regard to their efficacy to reduce the risk for dung or soil organisms and their practicability. For a more detailed discussion, please see sections 9.4.1 and 9.4.2; for an overview of the RMMs see Tables 29 and 32.

RMM: Strategic treatment of the animal group/herd is only allowed outside the periods of maximal abundance and diversity of dung organisms

Based on our current, limited knowledge on the biology and ecology of dung flies and dung beetles, it appears unlikely that appropriate time windows will be identified, during which dung organisms are inactive, so that parasiticides could be administered without harming dung organism communities. This is due to the fact that different dung organism species are active at different times of the year. Moveover, activity patterns depend on the geographic and climatic region, i.e. the RMM would need to be specified accordingly.

To evaluate the practicability of the RMM for cattle, sheep and horses, comprehensive data are needed on the usage of parasiticides, including information on the time / frequency of application for each parasiticide in the relevant live-stock animal species, breeds and age classes for each farming method / husbandry system. As emphasised at the project workshop, a detailed evaluation is required for each situation. Restrictions of the time, during which a parasiticide can be applied, have to be made for each livestock species and indication in close cooperation with parasitologists. In this context, possibilities to optimise the treatment regime should be evaluated. The RMM applies to strategic treatments of animal groups or herds. As suggested above, such treatments should be replaced by selective treatments or targeted selective treatments where possible. If, for example, young (first grazing season) animals are kept together with older animals, a selective treatment of the young animals could be performed. If the older animals remain untreated, dung without parasiticides would be available for dung organisms.

RMM: The product is toxic to dung organism (flies, beetles). Therefore, do not treat animals on the same pasture in successive seasons to avoid adverse effects on dung fauna and their predators

This risk mitigation measure is suitable to reduce the risk for multivoltine dung organism species. Whether univoltine dung organisms would benefit from the RMM, depends on the overlap of their reproductive cycles and the time of the antiparasitic treatment.

The RMM appears practicable for cattle, horses and sheep, if sufficiently large pasture areas are available, so that a rotational grazing scheme can be implemented. Whether the RMM can be implemented in routine farming practice, has to be evaluated for each specific situation. Compliance with the RMM is likely to be easy in rotational grazing systems with frequent rotation, but will probably be difficult or impossible in cattle and horse farms with limited pasture areas as was also stressed at the project workshop. In this context, it should also be mentioned that rotational grazing reduces the parasitic infection pressure on the pastures, leading to a win-win situation.

Again, targeted treatments appear to be an alternative to the RMM, since in this case both contaminated and uncontaminated dung is present on a pasture.

RMM: Animals from free-range husbandry must be stabled during treatment and for X days following treatment

To identify the duration of the stabling time required to protect dung organisms, more information is needed on the ecology of the most important dung organism groups, especially on their life-cycle characteristics, dispersal behaviour and recovery potential. Application of a safety factor of 100 to extrapolate from single-species laboratory tests to the whole dung organism community will probably not always be sufficient to protect the most sensitive dung organisms (cf. Blanckenhorn et al. 2013a). The required stabling time has to be specified for each parasiticide formulation / administration route, dose, pasture animal species and breed, given that these factors are influencing the excretion profile (section 7).

The RMM appears practicable in farming systems, where livestock is not kept on pastures all-year-round, if the period during which the animals have to be stabled is not too long and the pastures are relatively close to the stables. At the project workshop, it was highlighted that a longer time, during which the animals have to be stabled, might be in conflict with animal welfare requirements. For horses, the RMM is likely to be feasible, if the time, during which the animals have to be stabled, is relatively short. For cattle, practicability of this RMM is limited by the fact that the pastures are often not close to the stables. For sheep, the RMM will in most cases not be feasible. At the workshop, it was stressed that the RMM should not lead to a reduction of the percentage of livestock held on pastures.

In this context, it should be noted that stabling the animals during the period of peak excretion of the parasiticide would reduce exposure of dung organisms in the environment considerably, although this approach would not be sufficient to avoid effects on the most sensitive dung organisms (especially Sepsidae). However, it should also be considered that when the animals are stabled, their dung is not available to dung organisms, a fact that may also influence their abundance.

As for the two previously discussed RMMs, targeted treatments are considered as an alternative to this RMM, possible in combination with stabling of treated animals during the period of peak excretion, where feasible.

RMM: Manure from treated animals must be stored for X months prior to spreading on and incorporating into land to allow for degradation of the active substance prior to release into the environment

The RMM will protect soil organisms, if the prolonged period of manure storage results in a sufficient degradation of the respective parasiticide. Based on the DT_{50} of the active substance in stored liquid manure or dung of the relevant livestock animal species, the required duration of manure storage has to be specified for each parasiticide product. Yet, currently such DT_{50} values are not publicly available. The RMM can be applied to liquid manure or dung that is stored before spreading to land. Thus, it is relevant for cattle and horses, but in most cases not for sheep. Its practicability depends on the required storage time for liquid manure or dung of the respective farm animal species containing the parasiticide. A further, detailed evaluation has to be performed, when the DT_{50} values mentioned above have been generated.

RMM: When spreading liquid or solid manure from treated animals onto arable land, the maximum nitrogen spreading limit must not exceed X kg N per hectare and year (X < 170)

The measure will reduce local exposure to parasiticides and is thus suitable to protect the local soil organism communities. The reduced maximum nitrogen spreading limit has to be specified for each parasiticide product, livestock species, dosage, application frequency and manure-spreading scenario based on the risk quotient for soil organisms. This risk quotient should preferably be based on refined PEC_{soil} values, since for persistent parasiticides $PEC_{soil\ refined}$ may be higher than $PEC_{soil\ initial}$ (Liebig et al. 2010; see section 9.4.2.2). However, derivation of $PEC_{soil\ refined}$ is hampered by the lack of DT_{50} values for the parasiticide in stored manure (see above). In the absence of such values, $PEC_{soil\ initial}$ or, for persistent compounds, $PEC_{soil\ plateau}$ will need to be used instead. As the previous RMM, this measure can be

applied to manure spread to land, so that is relevant for cattle and horses, but generally not for sheep. In regions where farm animals are intensively kept, it might be difficult to find enough agricultural land where the manure could be spread. If the manure is sold, it has to be ascertained that information on the reduced maximum nitrogen spreading limit is passed on from the farmer selling the manure to the farmer applying the manure.

RMM: Manure containing the active substance should not be spread on the same area of land in successive years to avoid accumulation of the active substance, which may cause adverse effects on the environment

The RMM can reduce the accumulation of parasiticides in soil and, hence, protect the soil organism community. A consistent approach based on a $DT_{50\ soil}$ > 120 days, a $DT_{90\ soil}$ > 1 year and / or a $PEC_{soil\ plateau}$ / PNEC ratio ≥ 1 should be used to decide, if the measure should be implemented. Like the two previous measures, the RMM can be applied to manure spread to land and is thus relevant for cattle and horses, but not for sheep. In regions with intensive animal farming, its practicability is (as for the previous RMM) limited by the availability of sufficiently large agricultural areas to apply the manure. When the manure is sold, information on the antiparasitic treatment(s) has to be passed on from the farmer selling the manure to the farmer applying the manure.

Possibilities to restrict the use of authorised parasiticides

Overall, only a relatively limited number of antiparasitic products is available (see section 5). During the project workshop, it was pointed out that about 50% of the antiparasitic treatments of horses are carried out with macrocyclic lactones. Additionally, levamisole and benzimidazoles are used. To avoid the development of resistances, an alternating use of the different parasiticides is recommended. Cattle is mainly treated with macrocyclic lactones, while levamisole and benzimidazoles are only rarely used. In most cases, only first year animals are treated. Sheep are predominantly treated with macrocyclic lactones; as an alternative, levamisole is also used. In view of this small number of available parasiticides, the resistance situation (cf. section 6) and the limited perspectives for the development of new parasiticides (section 9.4.3), the replacement of an avermectin or milbemycin parasiticide by another active substance with similar efficiency but a reduced hazard and/or risk to the environment appears difficult. Since ivermectin, doramectin and eprinomectin have a similar toxicity to non-target organisms, the exchange of one of these parasiticides against another is unlikely to significantly reduce the risk for dung and, where a risk has been identified, soil organisms. Moxidectin has a lower toxicity than the three avermectins (cf. section 8). However, since it is bioaccumulative and thus fulfils the PBT criteria according to EC (2011), it appears no alternative to the three avermectins (see section 9.4.3).

10.4 Final considerations

In current livestock farming, parasiticides appear indispensable to effectively control parasitoses. Overall, their prudent use appears to be the most promising approach to reduce adverse effects on dung and soil organisms. A central point is to minimise the use of parasiticides by replacing strategic treatments by selective or targeted selective treatments where feasible. A collation and evaluation of data on the prevalence of parasites on farms, the usage of parasiticides, the success of antiparasitic treatments and the resistance situation could contribute to further develop case-specific approaches for an effective and sustainable control of parasites, combining prophylactic measures and optimised antiparasitic treatments. In this context, the work of the Belgian non-governmental organisation (NGO) NATAGRIVAL should be mentioned. This NGO informs and advises farmers, foresters and land owners in the implementation of agri-environmental measures and with regard to Natura 2000. The

work of NATAGRIVAL includes veterinary advice to farmers [28]. Focus is placed on a sustainable use of parasiticides considering animal health, the protection of the environment and economic aspects.

Risk mitigation measures may contribute to reducing the risk for dung and soil organism communities. Given that the risk quotients of parasiticides for dung organisms are generally much higher than those for soil organisms, main focus should be placed on further developing RMMs for the protection of dung organisms. At the project workshop, it was pointed out that several RMMs can also contribute to a reduction of resistances to parasiticides, thus leading to a win-win situation. However, for most of the evaluated RMMs substantial data gaps were identified that have to be closed to sufficiently specify the respective measure and to fully evaluate its suitability and practicability. Such an evaluation has to be performed for each parasiticide product and livestock species. Often, a further differentiation between livestock breeds and age classes, parasites, epidemiological situations and farming / husbandry systems is required. Generally, it should be pointed out that even if a measure can only be applied under certain conditions (depending e.g. on the farming method), it may still contribute to reducing the environmental risk.

The fact that the considered avermectins and moxidectin have the same (or a very similar) mode of action should be considered when specifying some of the evaluated RMMs. These RMMs should apply to all parasiticides with the same / a very similar mode of action. For instance, it it should be avoided to treat animals on the same pasture during successive seasons with different active ingredients having the same / a similar mode of action (see Tables 29 and 32).

At the project workshop, two further aspects were addressed:

First, it was suggested to verify if RMMs are in conflict with agri-environmental measures, e.g. provisions regarding delayed mowing or the frequent change of pastures in sheep husbandries (see e.g. Batáry et al. 2015). In case of conflicts of interests, the principal protection goal should be defined. There should be an overall concept for environmental protection on agricultural land. Moreover, it should be verified, if there are potential conflicts between RMMs and veterinary regulations.

Second, it was encouraged that the information exchange and cooperation between all involved parties (i.e. livestock owners / farmers, veterinarians, animal health services, pharmaceutical industry, competent authorities, environmental scientists) should be improved. An effort should be made to bring together basic research, applied research and veterinary / agricultural practice (see also section 11).

Last but not least, it should be pointed out that the current economic situation of farmers is a major factor limiting the practicability of a number of approaches that are outlined in the present report.

[28] See https://www.natagriwal.be/de/natagriwal/aktivitaeten.

11 Future perspectives

In this section, future perspectives in three areas related to the use, risk assessment and risk mitigation of parasiticides are outlined: (a) dung organism biology / ecology and the effects of parasiticides on dung organism communities, (b) sustainable approaches to control parasites, (c) risk mitigation measures, and (d) additional measures that are relevant for implementing new approaches into agricultural practice. The most important knowledge gaps that were identified in the present project are addressed.

Research needs regarding dung organism biology / ecology and the effects of parasiticides on dung organism communities

For almost all dung organisms, substantial information is lacking on the occurrence, ecology, life-cycle characteristics, dispersal behaviour, sensitivity to parasiticides and recovery potential (see sections 9.4.1.1 and 9.4.1.3). It is suggested to identify a central institution, which collects this information in a publicly available database. In addition, it should be pointed out that investigations of the diversity of beetle and, even more, fly communities are strongly hampered by the fact that only few specialists on the taxonomy of these organism groups are available. Therefore, it is of uttermost importance to improve the use of genetic methods for species identification. This includes the establishment of public databases, e.g. for barcoding (Blanckenhorn et al. 2016).

Only relatively few datasets from long-term field studies with parasiticides are publicly available. It has been shown that the use of the same parasiticide can cause different effects, e.g. on dung degradation, at different sites. Factors such as the time of the antiparasitic treatment, the species composition of the respective dung organism community and environmental conditions probably contribute to these differences (cf. sections 8.1, 8.3 and 10.2). Yet, additional research is needed to sufficiently understand why the use of the same parasiticide can cause different effects at different sites. Field studies would be helpful to further evaluate the consequences of antiparasitic treatments on functional (e.g. dung degradation) and structural (e.g. biodiversity) endpoints.

Open questions regarding sustainable approaches to control parasites

It is suggested that for each of the considered pasture animal species data on the prevalence of parasites in farms, the usage of parasiticides, the success of antiparasitic treatments, and the resistance situation in parasites should be collected and evaluated by a central institution. These data could then be used as a basis for further developing recommendations for the sustainable control of parasites combining optimised treatments with parasiticides and complementary approaches to control the parasites. Such recommendations could be provided as checklists or decision trees for farmers and veterinarians.

Currently used treatment frequencies should be critically checked and reduced where possible. Moreover, it should be verified where strategic treatments could be replaced by selective or targeted selective treatments. With regard to selective treatment approaches, there is a need to identify indicators that can be used to decide if a treatment is required and when this treatment should be performed (section 9.3). Further research is required to improve the diagnostics, especially with regard to practical and cost-effective methods, which can easily be applied in extensive cattle and sheep farming.

Considerable efforts are required to further develop possible alternatives to parasiticides such as vaccination, condensed tannins and the breeding of animals with an increased resistance to parasites. Further research is also needed to develop standardised methods for evaluating resistances to parasiticides (sections 6 and 9.3.4).

Further development and evaluation of risk mitigation measures

For most of the evaluated RMMs, data gaps were identified that have to be filled in order to sufficiently specify the measures and to fully evaluate their suitability and practicability (sections 9.4.1 and 9.4.2). Both, the specification and the subsequent evaluation of the RMM have to be made for each parasiticide product and livestock species. For many RMMs, a further differentiation between livestock breeds and age classes, parasites, epidemiological situations and farming / husbandry system will be necessary. In this context, it has to be stressed that for such an evaluation detailed data on the actual usage of the parasiticides are needed. As mentioned in the previous subsection, it is desirable to collect such data in a central register. Since most of the evaluated RMMs have the potential to contribute to a reduction of the environment risk caused by avermectins and milbemycins (Tables 29 and 32), it is recommended to further develop / specify and evaluate these measures.

A post-authorisation monitoring could be implemented to evaluate the efficacy of risk mitigation measures. Such a monitoring could especially be useful for parasiticides with PBT properties (see also Bänsch-Baltruschat et al. 2015). In this context, a targeted environmental monitoring, i.e. a long-term field study under farm conditions (cf. Römbke & Duis 2018), would be useful.

Additional measures relevant for implementing sustainable approaches into agricultural practice

As mentioned in section 10.4, only few data from long-term field studies with parasiticides are publicly available. This also applies to other (especially long-term) data on the environmental fate and ecotoxicity of parasiticides. The information published by the EMA in European public assessment reports [29] is still relatively limited. A public availability of the data used for the environmental risk assessment of parasiticides would e.g. allow to perform comparative assessments of different parasiticides, and is therefore highly desirable (see also Küster & Adler 2014).

As already stated in section 10.4, round tables with livestock owners / farmers, veterinarians, animal health services, pharmaceutical industry, competent authorities, environmental scientists and an intensive cooperation between all involved parties are essential for further developing sustainable approaches to control parasites and risk mitigation measures. If such a cooperation of all involved parties is successful, a reduction of the usage of antiparasitics is likely to be feasible for all considered pasture animal species. This would also help to avoid further resistances to parasiticides.

The workshop organised within this project was a first step in this direction, bringing people from all involved parties together. Such activities should to be organised on a regional level in order to ensure that scientific knowledge, practical experiences as well as economic and ecological needs are considered.

[29] See http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/general/general_content_000433.jsp&mid=WC0b01ac058067fa26

12 List of annexes

- Annex 1:

  Table 34: Overview of bioconcentration data for ivermectin and the related compound avermectin B_1 in fish

  Table 35: Overview of fish toxicity data for ivermectin and doramectin

- Annex 2:

  Summary of the workshop 'Risk management strategies for parasiticides used in pasture animals' (German Environment Agency, Dessau, Germany, 18-19 January 2017; in German)

  List of workshop participants

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14 Annex

14.1 Annex 1

This annex contains:

- Table 34: Overview of literature data on the bioconcentration of ivermectin and the related compound avermectin B_1 in fish.
- Table 35: Overview of literature data on the fish toxicity of ivermectin and doramectin.

The cited references are included in section 13.

Table 34: Overview of bioconcentration data for ivermectin and the related compound avermectin B_1 in fish. Additionally, information on bioconcentration of ivermectin in mussels is included. For both ivermectin and doramectin, no measured bioconcentration data are available for fish.

Test substance	Test organism	Test duration	Exposure system	Nominal conc.	Measured conc.	Bioconcentration factor	Remarks	Reference
Ivermectin								
Ivomec (0.5% ivermectin (w/v) in propanol)	Blue mussel (*Mytilus edulis*)	6 d uptake; 150 d depuration	Semistatic (daily renewal of water)	10 µg/L	6.9 µg/L	750 (on wet weight basis)	Steady state not reached. Estimated depuration half-life: 22 d (235°d)	Davies et al. 1997
Avermectin-B_1								
[^{3}H]-Avermectin-B_{1a}; purity: 99.5%	Bluegill sunfish (*Lepomis macrochirus*)	28 d uptake; 14 d depuration	Flow-through (6 volume exchanges / day)	0.1 µg/L	0.099 µg/L	52 (for whole fish, on wet weight basis) [a] 56 (for whole fish, on wet weight basis) [a]	Steady state on d 10-14	Wislocki et al. 1989 Van den Heuvel et al. 1996
Avermectin-B_1 (92% avermectin B_{1a}, 6% avermectin B_{1b}); purity: 98%	Sturgeon (species not specified)	22 d uptake; 18 d (0.2 µg/L) and 22 d (1.0 µg/L) depuration	Flow-through (5 volume exchanges / day)	0.2 and 1.0 µg/L	Only presented in a graph	42 (0.2 µg/L), 41 (1.0 µg/L), both for muscle, on wet weight basis	Steady state on d 14-18. Estimated depuration half-lifes: 5 d (0.2 µg/L), 4 d (1.0 µg/L)	Shen et al. 2005

Abbreviations: conc.: concentration(s); n.i.: not indicated

[a] Growth rate and percentage of lipid were not determined. Differences between the two reported values are due to differences in calculation (see van den Heuvel et al. 1996, p. 2264).

Table 35: Overview of fish toxicity data for ivermectin and doramectin. The table only includes studies with aqueous exposure.

Test substance	Test organism	Test method	Test duration	Nominal conc.	Measured conc.	Test result	Remarks	Reference
Ivermectin: acute toxicity data								
Ivermectin (Merck Sharp and Dohme); purity: n.i.	*Lepomis macrochirus*	Acute toxicity test according to U.S. EPA-660/3-75-009; solvent: dimethylformamide or triethylene glycol	96 h	n.i.	–	LC_{50}: 4.8 µg/L (extrapolated)	>50% effect in lowest test conc.	Halley et al. 1989a, b
Ivermectin; purity: n.i.		n.i.		n.i.	–	LC_{50}: 5.3 µg/L	Probably based on the test described by Halley et al. 1989a, b	Bloom & Matheson 1993
Ivermectin (Merck Sharp and Dohme); purity: n.i.	*Oncorhynchus mykiss*	Acute toxicity test according to U.S. EPA-660/3-75-009; solvent: dimethylformamide or triethylene glycol	96 h	n.i.	–	LC_{50}: 3.0 µg/L	–	Halley et al. 1989a, b
Ivermectin; purity: n.i.		n.i.	96 h	n.i.	–	LC_{50}: 3.3 µg/L	Probably based on the test described by Halley et al. 1989a, b	Bloom & Matheson 1993
Ivermectin (Ivomec: 1% ivermectin solution)	*Salmo salar*	Acute static test in seawater (30‰)	96 h	0.1 ng/L – 102 ng/L [a]	–	LC_{50}: 17 µg/L [a]	–	Kilmartin et al. 1996
Ivermectin (Ivomec: 1% ivermectin solution, M.S.D.-Agrivet)	*Anguilla anguilla*	Acute static test in seawater (28-30‰) with juvenile eels (15−30 g)	24 h	0.2−15 µg/L	–	LC_{50}: approx. 0.2 µg/L	The eels were infected with *Anguillicola crassus*	Geets et al. 1992
Ivermectin (Sigma) purity: 96.8%	*Danio rerio*	Acute toxicity test	96 h	n.i.	–	LC_{50}: 26 µg/L	–	B. Halling-Sørensen (pers. comm.)

Test substance	Test organism	Test method	Test duration	Nominal conc.	Measured conc.	Test result	Remarks	Reference
Ivermectin (Sigma); purity: 97%	*D. rerio*	Acute toxicity test (semi-static: daily exchange of test solutions) with adult zebrafish	96 h	10, 20, 40, 60, 80 and 100 µg/L	–	LC_{50}: 73 µg/L [b]	–	Domingues et al. 2016
Ivermectin: prolonged acute toxicity data								
Ivermectin (Sigma); purity: 97%	*D. rerio*	Prolonged acute test (semi-static: daily exchange of test solutions). Test endpoints: survival, growth, swimming behaviour, feeding behaviour, biomarkers (levels of vitellogenin, catalase, glutathione-S-transferase and cholinesterase)	21 d	0.25, 2.5 and 25 µg/L	–	At 25 µg/L. fish were lethargic / moribund Growth of males LOEC: 2.5 µg/L Growth of females LOEC: 25 µg/L Swimming behaviour LOEC: 0.25 µg/L Feeding behaviour LOEC: 2.5 µg/L	Effects on some biomarkers were recorded at 25 µg/L (vitellogenin in females, catalase in trunk tissue, glutathione-S-transferase in head tissue) and, partly, 2.5 µg/L (glutathione-S-transferase in head tissue), i.e. at concentrations severely affecting the general condition of the fish	Domingues et al. 2016
Doramectin								
Doramectin; purity: n.i.	*L. macrochirus*	Acute toxicity test	96 h	n.i.	n.i.	LC50: 11 µg/L	No information provided on test details	US FDA 2002
	O. mykiss	Acute toxicity test	96 h	n.i.	n.i.	LC_{50}: 5.1 µg/L		

[a] The derived LC_{50} of 17 µg/L is clearly outside the indicated range of test concentrations (0.1–102 ng/L). An error when reporting the units cannot be excluded. As in most of the other publications cited in this table, the results are only described in a very brief form. No concentration-response curve is provided.

[b] The LC_{50} is partly reported as 73 µg/L, partly as 73 mg/L. The former value is more plausible than the latter.

Abbreviations: conc.: concentration(s); n.i.: not indicated

14.2 Annex 2

This annex contains:

- A summary of the workshop 'Risk management strategies for parasiticides used in pasture animals' (German Environment Agency, Dessau, Germany, 18-19 January 2017; in German),
- A list of workshop participants.

Workshop

‚Ableitung von geeigneten Risikominderungsstrategien beim Einsatz von Antiparasitika in Weidetieren‘

18.-19.01.2017

Umweltbundesamt, Dessau-Roßlau

Zusammenfassung der Diskussion

Tag 1 (18. Januar 2017)

1. Einführung und Hintergrund

1.1. Hintergrund und Ziele des Projekts

(Referentin: Nicole Adler)

- In Hinblick auf die im Vortrag erwähnten Funde von Antiparasitika in Gülle, Boden/Sediment, Oberflächengewässer und Grundwasser wurde nachgefragt, welche Daten zu Antiparasitikanachweisen aus Deutschland stammen und wann (in welchen Jahren) die Nachweise erfolgt sind. Die von Arne Hein (Umweltbundesamt) zusammengestellten Daten stammen aus einer Literaturdatenbank, die mittlerweile auch über die Internetseiten des Umweltbundesamtes frei verfügbar ist[30]. Es handelt sich dabei um weltweite Einzelfunde (u.a. auch aus Deutschland) aus einzelnen Initiativmesskampagnen.
- Es wurde angesprochen, welchen Beitrag Tierarzneimittelprodukte für (a) Weidetiere bzw. allgemeiner Nutztiere und (b) Kleintiere (v.a. Hunde und Katzen) zum Gesamteintrag von Antiparasitika in die Umwelt leisten. Etwa 50% der Antiparasitikaprodukte sind für Lebensmittel liefernde Tiere (einschließlich Pferde) zugelassen, 50% für Kleintiere. Dies lässt allerdings keine Rückschlüsse auf die jeweiligen Anwendungsmengen zu. Makrozyklische Laktone sind überwiegend für Nutztiere zugelassen. In diesem Zusammenhang wurde erwähnt, dass Daten zu den Abgabemengen von Tierarzneimitteln (D, EU) von verschiedenen Marktforschungsunternehmen (z.B. kynetec, Vetnosis) erhoben werden und käuflich zu erwerben sind. Zudem werden Angaben zu den verkauften Mengen im Rahmen der regelmäßigen Berichte der Zulassungsinhaber zur Arzneimittelsicherheit (*Periodic Safety Update Reports*, PSUR), die den Zulassungsbehörden übermittelt werden, aufgeführt.

1.2. Aktueller Stand der Diskussion in der European Medicines Agency

(Referentin: Silke Hickmann)

- S. Hickmann wies darauf hin, dass die Aufnahme von Risikomanagementmaßnahmen in die Zusammenfassung der Merkmale des Tierarzneimittels (*Summary of Product Characteristics, SPC*) verpflichtend ist, wenn ein mögliches Risiko identifiziert wird. Die Umsetzung der Risikomanagementmaßnahmen durch den Adressaten ist jedoch nicht rechtlich verbindlich. Eine vergleichende Bewertung verschiedener Tierarzneimittelwirkstoffe wäre sinnvoll, es gibt allerdings bisher keine Vorgaben, wie eine solche Bewertung erfolgen sollte. In anderen Stoffrechten, wie

[30] Siehe https://www.umweltbundesamt.de/en/database-pharmaceuticals-in-the-environment-0

dem Pestizidrecht, ist die vergleichende Bewertung verankert. Für als PBT klassifizierte Wirkstoffe könnte eine zeitlich begrenzte Zulassung eine Option sein. Bei einer negativen Nutzen-Risikobewertung könnte ein *phase-out* eine Option sein. Längerfristig werden klarere Regelungen zur Bewertung von PBT-Substanzen benötigt.

- In Hinblick auf mögliche Resistenzen wurde angeregt, dass Informationen zu einer fehlenden Wirksamkeit einer antiparasitären Behandlung häufiger als bisher an die Behörden weitergeleitet werden sollten. In diesem Zusammenhang wurde die Einrichtung bzw. Identifikation von Referenzlaboren angeregt, von denen Angaben zur Effizienz von Behandlungen und zur Resistenz gegenüber Wirkstoffen erhoben bzw. gesammelt werden sollten. Es wurde angemerkt, dass es im Gegensatz zu Antibiotika bis jetzt keine standardisierten Prüfmethoden und Schwellenwerte für die Resistenzbestimmung bei Antiparasitika gibt. In diesem Zusammenhang wurde auf das *Reflection paper on anthelmintic resistance* (CVMP/EWP/573536/2013) hingewiesen. Generell wurde angemerkt, dass der Datenaustausch zwischen Anwendern, Tierärzten und Behörden zurzeit nicht optimal ist. Außerdem ist die Verschickung von Proben an Labore, die Resistenzuntersuchungen durchführen, zum Teil technisch schwierig.

2. Ergebnisse der experimentellen Arbeiten

2.1. Oktanol/Wasser-Verteilungskoeffizienten für Ivermectin und Selamectin

(Referentin: Monika Herrchen)

- Es wurde ein anderes Antiparasitikum angesprochen, bei dem bei einer Verlängerung der Equilibrationszeit über 72 h hinaus ein geringerer log P_{ow}-Wert ermittelt wurde. Das konnte darauf zurückgeführt werden, dass sich die Verteilung nach mehr als 72 h zugunsten der Wasserphase verschoben hat. Über die Bedeutung dieser Beobachtung sowie deren mögliche Verallgemeinerung kann jedoch keine Aussage getroffen werden.

2.2. Biokonzentration von Ivermectin und Doramectin in Fischen

(Referentin: Karen Duis)

- Da Avermectine stark an organische Substanzen adsorbieren, ist damit zu rechnen, dass Organismen in der Umwelt sie mit der Nahrung aufnehmen. Vor diesem Hintergrund wurde nach der erwarteten Anreicherung in Fischen bei Aufnahme mit der Nahrung gefragt. Im vorliegenden Projekt wurden Biokonzentrationsfaktoren (Anreicherungsfaktoren nach Exposition über das Wasser) ermittelt. Eine mögliche Umrechnung von Biokonzentrationsfaktoren in Biomagnifikationsfaktoren (Anreicherungsfaktoren nach Exposition über die Nahrung) wird zurzeit diskutiert (u.a. auf OECD-Ebene). Vorliegende Studien mit Fischen zur Aufnahme von Ivermectin über die Nahrung zielten darauf ab, Eliminationszeiten (und darauf basierend Wartezeiten nach Verabreichung an Fische) zu ermitteln. In sedimentbewohnenden Würmern wurden Biota-Sediment-Akkumulationsfaktoren von bis zu 5,5 ermittelt.
- Es wurde z.T. mit Erstaunen festgestellt, dass so zentrale Daten zur Umweltrisikobeurteilung von Wirkstoffen, die seit Jahrzehnten angewendet werden, nicht schon längst vorliegen. Für eine Reihe von alten Wirkstoffen wurden entsprechende Studien aber bereits durchgeführt.
- Es wurde darauf hingewiesen, dass die ermittelten BCF-Daten deutlich unter dem Schwellenwert von 2000 für die Klassifikation von Substanzen als bioakkumulierend liegen.

3. Ergebnisse der Literaturrecherchen

3.1. Exkretion von Avermectinen und Milbemycinen durch Weidetiere

(Referent: Ludwig Hölzle)

- Es wurde auf Unterschiede im Stoffwechsel und demzufolge in der Exkretion von Parasitiziden zwischen verschiedenen Rinderrassen (v.a. Milch- vs. Fleischrinder) hingewiesen. Die Exkretion hängt außerdem vom Alter und Geschlecht der Tiere und von der eingesetzten Wirkstoff-Formulierung ab.
- Generell wurde mit Verweis auf z.B. EMA-Dossiers hinterfragt, ob die Datenlage wirklich so schlecht ist. Aufgrund dieses Hinweises wird eine erneute Überprüfung entsprechender Dossiers durchgeführt werden.

3.2. Effekte von Avermectinen und Milbemycinen auf Dungorganismen

(Referent: Jörg Römbke)

- Die Relevanz der vorgestellten Daten zu den Effekten von Avermectinen und Milbemycinen auf Dungorganismen wurde kontrovers diskutiert. Das sog. *meadow fouling* kann so stark ausgeprägt sein, dass es negative Auswirkungen auf die Nutzung der betroffenen Flächen hat, da z.B. Rinder mit Dung bedeckte Flächen beim Weiden meiden (vgl. z.B. Anderson et al. 1984, J Econ Entomol 77, 33-141). Der Zusammenhang zwischen der Toxizität von Antiparasitika auf Dungorganismen und den Abbauraten von Dung ist bisher allerdings unzureichend erforscht. Es wurde auf Feldstudien hingewiesen, in denen keine signifikanten Effekte von Antiparasitika auf den Dungabbau gezeigt werden konnten. Nach jetzigem Kenntnisstand gibt es dafür keine generell gültige Begründung. Unterschiede in der Exposition, dem Zeitpunkt der Behandlung (und damit der Aktivität der Dungorganismen) sowie die Zusammensetzung der jeweiligen Dungabbauenden Organismengemeinschaft spielen sicher eine Rolle. Es gibt allerdings keine Studien, in denen nach Verwendung von Antiparasitika keine Effekte auf Dungorganismen (vor allem Dungfliegen und deren Larven) gefunden wurden. Damit ist ein Risiko für das Schutzziel Biodiversität der Dungorganismengemeinschaft gegeben.
- In diesem Zusammenhang wurde auch danach gefragt, wie schnell Dunghaufen wiederbesiedelt werden. Dieser Vorgang wird u.a. dadurch begrenzt, dass viele Dung-bewohnende Arten an eine bestimmte Kotbeschaffenheit angepasst sind, d.h. ein begrenztes *window of opportunity* haben. So können z.B. einige Arten den Kot nicht mehr besiedeln, wenn die Kotoberfläche zu fest ist. Fehlt eine Art, die Gänge in den Dunghaufen anlegt, können andere, von diesem Vorgang abhängige Arten diese Dunghaufen ebenfalls nicht bearbeiten.
- Es wurde angemerkt, dass die Behandlung mit Antiparasitika nicht immer zum gleichen Zeitpunkt bzw. flächendeckend erfolgt. Dadurch sowie durch die Koexistenz von Wildtieren mit Weidetieren sollten in gewissem Umfang alternative Nahrungsquellen (d.h. Kot ohne Antiparasitika) für Dungorganismen vorhanden sein. Viele – wahrscheinlich die meisten – Dungorganismen sind allerdings auf bestimmte Dungquellen angewiesen, so dass ein Wechsel von Nutz- zu Wildtierdung nur in sehr eingeschränktem Maß möglich ist (bekanntestes Beispiel in Deutschland: Bindung einer Käferart an den Kot von Feldhamstern). Zudem haben viele Dungorganismenarten eine zeitlich eng begrenzte Reproduktionsphase. Größere Dungkäfer sowie die eher kleinen Arten der sehr artenreichen Gattung *Aphodius* bilden nur eine Generation pro Jahr aus. Zum Beispiel vermehrt sich die Standardtestspezies *Aphodius constans* nur im Zeitraum Januar bis März, mit leichten Schwankungen je nach Region und Höhenlage. Aus der Literatur ist

bekannt, dass bestimmte Fliegenarten sich nicht fortpflanzen können, wenn sie in diesem Zeitfenster keinen Dung eines bestimmten Alters (d.h. einer bestimmten Beschaffenheit) finden.

- In der Diskussion wurde darauf hingewiesen, dass Dunghaufen auch durch Starkregenereignisse zerstört werden. Dieser Prozess ist allerdings nicht mit Abbau zu verwechseln: Starkregen bewirkt stattdessen, dass dem Ökosystem Nährstoffe entzogen werden (z.B. durch Oberflächenabfluss), die dann für Dung- und Bodenorganismen sowie Pflanzen fehlen.
- Hinsichtlich der Erfassung der Diversität von z.B. Dungkäferarten wurde auf die Möglichkeit der genetischen Artbestimmung (*Barcoding*) verwiesen. Dabei werden ausgewählte DNA-Sequenzen anhand kleiner Proben von einzelnen Individuen zur Artbestimmung verwendet. Erste Erhebungen, z.B. durch Prof. Blanckenhorn (Universität Zürich), lieferten robuste Ergebnisse, doch ist aufgrund unzureichender Vergleichsdaten die Verbindung zur „klassischen" Taxonomie sowie zu der damit verknüpften Ökologie dieser Tiere noch ausbaufähig. Daher ist es notwendig, regional differenzierte Vergleichsdatenbanken für die wichtigsten Dungorganismenarten anzulegen.
- Abschließend fragte Jörg Römbke die Tagungsteilnehmer nach ergänzenden Daten, die dem Projektkonsortium eine bessere Einschätzung der gesamten Problematik ermöglichen könnten.

Tag 2 (19. Januar 2017)

4. Risikomanagementstrategien für Avermectine und Milbemycine

4.1. Einführung (Hintergrund, Definitionen, Praktikabilität)

(Referentin: Nicole Adler)

- Einige Risikominderungsmaßnahmen für Antiparasitika könnten gleichzeitig zu einer Reduktion der Resistenzproblematik und damit zu einer *win/win*-Situation führen.
- Die Informationen in der Zusammenfassung der Merkmale des Tierarzneimittels (SPC) können für ein neu zugelassenes Tierarzneimittel anders sein als für ein Altpräparat mit demselben Wirkstoff. In Hinblick auf eine Harmonisierung der Risikomanagementmaßnahmen innerhalb der EU wurde angeregt, dass die entsprechenden Informationen im SPC wo nötig angepasst / spezifiziert werden sollten. Eine verpflichtende Anpassung von SPCs ist jedoch nur bei schwerwiegendem Anlass im Rahmen von Schiedsverfahren (*referrals*) möglich.
- Zurzeit werden die meisten Antiparasitika in europäischen Verfahren zugelassen, etwa 2/3 der zugelassenen Präparate sind Generika.
- Das aktuelle Pharmakovigilanzsystem für Tierarzneimittel eignet sich i. Allg. nicht zur Erfassung von Auswirkungen auf die Umwelt, da nur auffällige Ereignisse erfasst werden, während allmähliche Veränderungen und Auswirkungen auf „unauffällige" Organismengruppen meist nicht erkannt werden.
- Seitens des Gesetzgebers gibt es kaum Hilfestellungen bei der Einführung bzw. Umsetzung von Risikominderungsmaßnahmen. Die Einhaltung von Risikominderungsmaßnahmen wird nicht überwacht, d.h. es gibt auch keine Sanktionen.
- In der Diskussion wurde darauf hingewiesen, dass Informationen zu Risikominderungsmaßnahmen in Packungsbeilagen für verschiedene Präparate mit demselben Wirkstoff nicht durchgängig vorhanden sind. Allerdings seien sie – z.B. abhängig von der Formulierung des jeweiligen Produktes – auch nicht bei jedem Präparat notwendig. Die geringe Akzeptanz von Risikominderungsmaßnahmen könnte auch dadurch bedingt sein, dass für Laien auffällige Wirkungen von Antiparasitika in der Umwelt eher selten sind.

4.2. Nachhaltige Herangehensweisen zur Kontrolle von Parasiten: Optimierung von Behandlung, Weidemanagement und Haltung, alternative Methoden zur Parasitenbekämpfung

(Referent: Ludwig Hölzle)

- In der Diskussion wurde mehrfach darauf hingewiesen, dass bei der Diskussion der Risikominderungsmaßnahmen generell zwischen den jeweiligen Nutztierarten und Haltungsformen differenziert werden muss.
- Eine Behandlung vor dem Weideaustrieb würde zu einer Verringerung der Exposition von Dungorganismen führen. Sie ist jedoch bei hoher Parasitenlast auf der Weide nicht optimal. Außerdem werden v.a. erstsömmrige Rinder mit Antiparasitika behandelt. Diese sind zum Zeitpunkt des Weideaustriebs noch parasitenfrei. Aufgrund der Entwicklung der Parasitenpopulation und der Befallsrate der Tiere wäre aus veterinärmedizinischer Sicht eine Behandlung ca. 6-8 Wochen nach dem Weideaustrieb optimal[31].

[31] Die Wirkung der Antiparasitika hält etwa 2-3 Wochen an.

- Während der Weidephase ist eine Einstallung nach einer antiparasitären Behandlung oft nicht praktikabel (siehe auch Abschnitt 4.3 dieses Annexes).
- Es wurde angemerkt, dass Impfstoffe gegen den Lungenwurm beim Rind in Deutschland nicht mehr verfügbar sind und dass Impfungen in den nächsten 10 bis 20 Jahren vermutlich keine relevante Alternative zur Behandlung mit Antiparasitika sein werden.
- Zur Bekämpfung von Parasitenstadien können Weiden mit Kalkstickstoff behandelt werden. Vorliegende Ergebnisse zur Effektivität dieser Maßnahme divergieren jedoch stark.
- Eine Kontrolle der Wirksamkeit von antiparasitären Behandlungen ist wünschenswert; in diesem Bereich sollten Verbesserungen angestrebt werden.
- Es sollte überprüft werden, ob vorgeschlagene Risikominderungsmaßnahmen mit Agrarumweltmaßnahmen (z.B. Vorschriften zum Zeitpunkt der Mahd und zur Wechselbeweidung in der Schafwirtschaft) in Konflikt stehen. Bei vorliegenden Interessenskonflikten (z.B. Vogelschutz / Pflanzenschutz / Dungorganismenschutz) muss das Hauptschutzziel definiert werden. Es sollte ein Gesamtkonzept für den Umweltschutz auf landwirtschaftlichen Flächen geben.
- Auch mögliche Konflikte mit veterinärrechtlichen Vorschriften (z.B. zur Bekämpfung von Überträgern der Blauzungenkrankheit, Schweinehaltungshygieneverordnung) sollten überprüft werden.
- Es wurde angeregt, deutlicher zwischen Ekto- und Endoparasitenmitteln zu differenzieren. Da es kaum noch *pour-on*-Präparate auf dem Markt gibt, ist die Unterscheidung zwischen der Bekämpfung von Endo- und Ektoparasitenmitteln in Hinblick auf den Eintrag des Wirkstoffs in die Umwelt und geeignete Risikominderungsmaßnahmen jedoch weniger relevant als früher.
- Es wurde angemerkt, dass es für Schafe zurzeit kein zugelassenes Ektoparasitenmittel gibt. Laut Vetidata sind jedoch mehrere Produkte (z.B. zur Behandlung von Zecken, Läusen, Schaflausfliegen) zugelassen.
- Es wurde auf die Bedeutung der Beratung des Tierhalters durch den Tierarzt hingewiesen (auch im Bereich der Kleintiere, d.h. v.a. Katzen und Hunde).
- Die Vorgehensweise der gezielten selektiven Behandlung von Nutztieren ist oft bekannt und wird in einem Teil der landwirtschaftlichen Betriebe angewandt. Es besteht allerdings noch Forschungsbedarf hinsichtlich der Behandlungsindikatoren: wann ist eine Behandlung nötig, weil das Wohlbefinden / die Leistung des Tieres beeinträchtigt ist?
- In anderen Ländern werden bereits gezielt wurmtolerante Nutztiere gezüchtet. So werden z.B. in der Schweiz Schafböcke eine Saison lang ohne antiparasitäre Behandlung auf der Weide gehalten (sog. Bockweiden). Die für die Zucht verwendeten Schafböcke werden u.a. anhand der anschließend bestimmten Anzahl Eier pro Gramm Kot (EpG) ausgewählt. Es wurde angemerkt, dass solche Zuchtprogramme staatlich gefördert werden müssten.
- Abschließend wurde nochmals betont, wie wichtig die Zusammenarbeit mit den jeweiligen Tierhaltern ist und dass diese durchaus noch intensiviert werden könnte.

4.3. Risikomanagementmaßnahmen zum Schutz von Dungorganismen: Einsatz von Antiparasitika außerhalb der Aktivitätszeit von Dungorganismen, nicht jedes Jahr auf derselben Weide, Stallhaltung während und nach der Behandlung

(Referent: Jörg Römbke)

a) Strategische Behandlungen von Tiergruppen bzw. ganzen Herden nur außerhalb der Populations- bzw. Diversitätsmaxima von Dungorganismen

– Um diese Risikomanagementmaßnahme ausreichend spezifizieren zu können, sind weitere Vorarbeiten nötig.

b) Produkt ist toxisch für Dungorganismen. Deshalb dürfen behandelte Tiere nicht jede Saison auf derselben Weide gehalten werden

– Es wurde darauf hingewiesen, dass diese Risikomanagementmaßnahme für die verschiedenen Nutztierarten getrennt diskutiert werden sollten (das gilt auch für einige andere Maßnahmen). So werden in Schafbetrieben mit Wechselbeweidung Flächen oft nur 1 Mal pro Jahr beweidet. Welche Weide wann beweidet wird und wann die Tiere mit Antiparasitika behandelt werden, wird im Weidetagebuch verzeichnet. Aufeinanderfolgende Behandlungen erfolgen normalerweise nicht auf derselben Fläche. In der Rinder- und Pferdehaltung werden hingegen oft in aufeinanderfolgenden Jahren die gleichen Weiden genutzt. Zusätzliche Weiden stehen nicht zur Verfügung, so dass die Maßnahme nicht praktikabel ist. Hier wäre evtl. ein Wechsel der Beweidung durch behandelte / unbehandelte Tiere innerhalb eines Jahres eine Option.

c) Tiere in Freilandhaltung während Behandlung und während der nächsten X Tage nach der letzten Verabreichung im Stall halten

– Auch hier wurde eine separate Diskussion für die verschiedenen Nutztierarten angeregt. So ist z.B. bei der Behandlung von Pferden gegen Spulwürmer eine Einstallung aus veterinärhygienischer Sicht sinnvoll. Diese Einstallung beschränkt sich aber meist auf 2-3 Tage. Eine längere Einstallung könnte in Hinblick auf das Tierwohl problematisch sein. Rinderweiden sind hingegen oft so weit von den Ställen entfernt, dass eine Einstallung nach einer antiparasitären Behandlung während der Weidephase in vielen Fällen nicht praktikabel ist. Die diskutierte Risikomanagementmaßnahme sollte nicht zu einer Abnahme der Weidehaltung von Rindern führen.

– Alternative Wirkstoffe zur Behandlung von Endoparasiten wurden diskutiert. In diesem Zusammenhang wurde auch auf an der FU Berlin erstellte Dissertationen hingewiesen.

  – Bei Pferden werden ca. 50% der antiparasitären Behandlungen mit makrozyklischen Laktonen durchgeführt. Außerdem werden Levamisol und Benzimidazole eingesetzt. Um die Entstehung von Resistenzen zu vermeiden, wird ein Wechsel des Wirkstoffs in aufeinanderfolgenden Behandlungen empfohlen.

  – Bei Rindern werden hauptsächlich makrozyklische Laktone eingesetzt. Levamisol wird selten, Benzimidazole werden sehr selten verwendet. In der Regel werden nur die erstsömmrigen Tiere behandelt.

  – Auch bei Schafen werden überwiegend makrozyklische Laktone verwendet, alternativ Levamisol. Benzimidazole sind wegen der verbreiteten Resistenzen keine gute Alternative (wenn sie verwendet werden, dann v.a. für Mastlämmer).

– Ein Stufenprinzip für Risikomanagementmaßnahmen wurde angeregt: eine Maßnahme müsste dann von Betrieben umgesetzt werden, die mehr als 1, 2 oder 3 Mal pro Jahr entwurmen.

- Es wurde gefragt, ob in den Feldstudien mit Ivermectin, die in dem im Vortrag erwähnten vom UBA geförderten Projekt durchgeführt wurden, die Besiedlung des Dungs auch an einem späteren Zeitpunkt (z.B. nach 3 Monaten) untersucht wurde. Eine solche Untersuchung konnte im Rahmen des Vorhabens leider nicht durchgeführt werden.

4.4. Risikomanagementmaßnahmen zum Schutz von Bodenorganismen (1): Lagerung von Dung/Gülle von behandelten Tieren vor dem Ausbringen

(Referentin: Monika Herrchen)

- Da keine belastbaren Daten zum Abbau von Antiparasitika in Gülle und Dung vorliegen, kann zurzeit nicht abgeschätzt werden, für wie viele / welche Wirkstoffe eine vorgegebene Mindestlagerzeit infrage kommt.

4.5. Risikomanagementmaßnahmen zum Schutz von Bodenorganismen (2): Begrenzung der auszubringenden Dung- bzw. Güllemenge, Ausbringung nicht jedes Jahr auf dieselbe Fläche

(Referentin: Karen Duis)

- In Regionen mit intensiver Tierhaltung ist die Güllemenge / ha und dementsprechend auch der potenzielle Eintrag von Tierarzneimitteln hoch. Es wird erwartet, dass die neue Düngeverordnung hier Verbesserungen für die Umwelt bewirken wird.
- Es wurde darauf hingewiesen, dass die Schlussfolgerung, Risikomanagementmaßnahmen sollten primär auf den Schutz von Dungorganismen fokussieren, nur die im Vorhaben betrachteten Antiparasitika (und den Vergleich von Maßnahmen zum Schutz von Boden- und Dungorganismen) betrifft.

4.6. Sind Anwendungsbeschränkungen für einzelne Wirkstoffe eine effektive und praktikable Option? Gibt es weitere Ideen?

(Referent: Jörg Römbke)

- Die Effekte auf Dungorganismen wurden in den meisten Fällen nach Verabreichung von Antiparasitika an Rinder untersucht. Für andere Nutztierarten liegen deutlich weniger Daten vor.

5. Diskussion der vorgestellten Risikomanagementmaßnahmen und ggf. Anwendungsbeschränkungen

- Im Bereich des Risikomanagements von Antiparasitika sollte die Interaktion zwischen den verschiedenen Akteuren verbessert werden.
- Bei der Formulierung von Risikomanagementmaßnahmen für Antiparasitika sollten Aussagen zum Anwendungszeitpunkt für jede Tierart (Minimum: Rind, Schaf, Pferd) und Indikation spezifiziert und in Rücksprache mit Parasitologen gemacht werden.
- Es wurde angemerkt, dass der Aspekt ‚Biologie der Parasiten' auf dem vorliegenden Workshop gefehlt hat.
- Generell sollte das Auftreten klinisch manifester Effekte von Parasiten vermieden werden (d.h. es sollte nicht erst behandelt werden, wenn diese Effekte auftreten). Es wurde betont, dass ein genereller Verzicht auf Antiparasitika nicht möglich ist.
- Allgemein wurden der richtige Anwendungszeitpunkt und ein umsichtiger Einsatz von Antiparasitika als Schlüsselfaktoren identifiziert.

- Es wurde nochmals auf die notwendige Differenzierung zwischen verschiedenen Nutztierarten und Haltungsformen hingewiesen. Die meisten hier diskutierten Maßnahmen sind v.a. für die intensive Rinderwirtschaft relevant.
- In Hinblick auf Exkretionsdaten wurde auf öffentlich verfügbare Dossiers (u.a. zu *maximum residue limits*) und (*European*) *public assessment reports* hingewiesen.
- Konkrete Rückmeldungen der Workshop-Teilnehmer zu ergänzenden Daten wären sehr hilfreich.

6. Zusammenfassung und Ausblick

(Nicole Adler)

Zusammenfassend kann gesagt werden, dass in Hinblick auf Risikomanagement und Risikominderungsmaßnahmen folgende Aussagen und Anmerkungen gemacht wurden:

- Da zurzeit keine Impfstoffe verfügbar sind, sind keine Alternativen zu Antiparasitika vorhanden.
- Im Bereich Diagnostik / Behandlungsindikatoren gibt es Forschungsbedarf. Hier wären entsprechende Fördermaßnahmen sinnvoll. Geeignete Behandlungsindikatoren sind die Voraussetzung für eine gezielte selektive Behandlung von Tieren.
- Ein Wechsel von Wirkstoffen ist generell möglich, hier ist jedoch eine differenzierte Betrachtung notwendig.
- Der Tierhalter sollte bei den Maßnahmen mehr im Blick gehalten werden.
- Die Kommunikation mit Tiergesundheitsdiensten sollte verstärkt werden.
- Es wurde festgestellt, dass Agrarumweltmaßnahmen und Risikominderungsmaßnahmen z.T. miteinander in Konflikt stehen könnten, hier sollte ein Umweltgesamtkonzept erarbeitet werden.
- Einige der formulierten Maßnahmen (z.B. Hygienemaßnahmen) sind in der Tierhaltung selbstverständlich.
- Es wurde festgestellt, dass die auf dem Workshop betrachteten Daten zu sehr auf die intensive Rinderwirtschaft fokussiert sind. Eine Differenzierung von Maßnahmen in Hinblick auf die behandelte Tierart, die Indikation sowie die Art und Anzahl der Behandlungen ist notwendig und wahrscheinlich auch zielführender.
- Für den Schutz der Dungfauna ist der Behandlungszeitraum wichtig. Hier müssten – soweit möglich – geeignete Zeitfenster für Behandlungen in enger Abstimmung mit Parasitologen ermittelt werden.
- Sowohl Parasiten als auch Dungfauna müssen betrachtet werden, das geht nur durch eine bessere Kommunikation auf beiden Seiten.
- Es besteht der Wunsch, dass Datenlücken geschlossen werden, z.B. durch das Zugänglichmachen von vorhandenen Daten. Eine Zusammenfassung vorliegender Daten könnte die Basis für eine Verbesserung des Risikomanagements sein.

Weiterer Forschungsbedarf wurde in folgenden Bereichen identifiziert:

- Übertragbarkeit von Labortestergebnissen auf das Freiland,
- Freilandtests mit anderen Wirkstoffen als Ivermectin,
- Effekte von Wirkstoffwechseln,

– Zusammenführen der Grundlagenforschung mit der angewandten Forschung und der landwirtschaftlichen / tierärztlichen Praxis.

Participants of the workshop

'Risk management strategies for parasiticides used in pasture animals'

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