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Presence of pesticides and biocides at Dutch cattle farms participating in bird protection programs and potential impacts on entomofauna

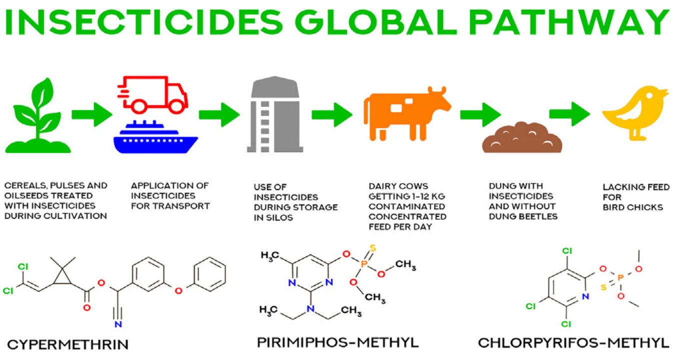
Jelmer Buijs [a,b,*], Ad Ragas [b], Margriet Mantingh [c]

[a] Buijs Agro-Services, Schuurhoven 19, Bennekom 6721SM, the Netherlands
[b] Department of Environmental Science, Radboud Institute for Biological and Environmental Sciences, Faculty of Science, Radboud University, Nijmegen, the Netherlands
[c] WECF, Korte Elisabethstraat 6, Utrecht 3511 JG, the Netherlands

HIGHLIGHTS

- 129 different pesticides found on 23 cattle farms
- 69 pesticides found on 8 certified organic cattle farms and 115 at 15 conventional farms
- Negative correlation between total insecticide uptake by cows and Coleoptera counts in cow dung
- Pesticide concentrations in cattle manure of organic farms 43% lower than of conventional farms
- No difference found in the numbers of Coleoptera in manure of organic and conventional farms

GRAPHICAL ABSTRACT



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ABSTRACT

In spite of meadow bird protection programs, a severe decline of meadow birds is taking place in the Netherlands. It is hypothesized that pesticides and other agrochemicals may contribute to this decline through a negative impact on the entomofauna; a very important food source of meadow birds and especially of their chicks. The present study analysed the presence of 664 pesticides (including biocides and some metabolites) in soil, concentrated feed, manure and some fodder samples from 23 cattle farms in the province of Gelderland (the Netherlands). Furthermore, the presence of 21 anti-parasitic medicines in manure from storage facilities was analysed. For farms practicing field grazing, the number of dung beetles in field samples of fresh manure was determined and a potential relationship with the presence of pesticide residues was explored. Of the 23 farms included in present study, 22 participated in meadow bird protection schemes. A total of 129 different pesticides (including biocides and metabolites) was detected, of which 115 at the 15 conventional farms and 69 at the 8 certified organic farms. The average total amount of pesticide residues detected tended to be lower at organic cattle farms than at conventional farms; for organic concentrated feed this difference was significant at a factor of 3.7. A significant negative correlation was found between the estimated daily intake of insecticides by cattle through the consumption of concentrated feed and hay, and the numbers of dung beetles detected in fresh manure samples in the field. We discuss the most important insecticides detected in concentrated feed and hay, and conclude that their quantities in manure and feed, if compared with LR50 values, give a reason for concern. More research is needed to establish the role of agrochemicals in the decline of meadow birds.

1. Introduction

Animal husbandry is a traditionally strong economic sector in the Netherlands (Afrian et al., 2020). Its main produce are dairy products,

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* Corresponding author at: Buijs Agro-Services, Schuurhoven 19, Bennekom 6721SM, the Netherlands.
E-mail address: jelmer.buijs@ru.nl (J. Buijs).

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meat and eggs. Over the centuries, the traditional way of farming, especially dairy farming, resulted in a landscape which was rich in meadow birds (Beintema et al., 1995; Kruk, 1994), with densities of lapwings and black-tailed godwits reaching 1 breeding pair per ha or more (Heijnis, 1973). In more recent decades, a dramatic and steady decline in populations of meadow birds has been observed; not only in the Netherlands, but also in other EU member states (Roodbergen and Teunissen, 2014) and in North America (Rosenberg et al., 2019).

Various studies have indicated that meadow birds in the Netherlands suffer, among others, from the modern management of agricultural grasslands which is characterized by lowered water tables, frequent mowing, pastures of uniform grass (lacking herbs), high cattle stocking density, injection of liquid manure and an increased abundance of predators (Verhulst et al., 2007; Franks et al., 2018; Schröder, 2010; Trouwborst, 2016; Kenty et al., 2014). This has triggered the Dutch government and bird protection societies to establish bird protection areas (Verhulst et al., 2007; Roodbergen and Teunissen, 2014). However, in spite of protective measures like delayed mowing and nest protection, very few bird chicks become full-grown (Van der Vliet et al., 2015; Roodbergen et al., 2018). The breeding success is insufficient to re-establish past population levels or to conserve present population levels, with the consequence that most populations of breeding meadow birds show a steady decline (Kleijn and Van Zuijlen, 2004; Roodbergen and Teunissen, 2014).

Chicks of meadow birds like black tailed godwits and lapwings predominantly feed on insects and other arthropods (Beintema et al., 1991; Schröder, 2010). Reduced food availability for meadow bird chicks has been identified as one of the main drivers of the decline in reproductive success (Vickery et al., 2001). This reduced availability of insects and other arthropods can be triggered by several factors such as a reduction in plant species richness and an increased vegetation density in agricultural grasslands (Crawley et al., 2005; Wiggers et al., 2016). Additionally, the grass harvesting process can have a substantial negative impact on invertebrate populations (Humbert et al., 2009). Reductions in insect abundance have also been noted beyond agricultural grassland. For example, in German nature reserves, the biomass of flying insects declined by about 76% from 1989 until 2016 (Hallmann et al., 2017). A similar decline of beetles was observed in Dutch nature reserves (Hallmann et al., 2020). A recent review by Raven and Wagner (2021) shows that this decline in insect biodiversity is a global phenomenon, related to changing land use, agricultural intensification and climate change. A potential contributing factor is the widespread use of pesticides in agriculture and their subsequent dispersal into the environment. In the USA, pesticides have been reported to be the most dominant reason for bird decline (Stanton et al., 2018). A review by Bright et al. (2008), focusing on the UK, concluded that pesticides act mainly through a reduction in food supplies.

A potential route through which pesticides may affect insect and arthropod abundance in grasslands that has received relatively little attention from the scientific community, is the direct and indirect use of pesticides and other chemicals, such as veterinary drugs, in animal husbandry. Considerable amounts of pesticides are for example being used in the cultivation of crops from which concentrated feed is being produced which is subsequently fed to the animals. Also, during the storage and transport of feed, considerable amounts of pesticides are being used (CEEU, 2018). Veterinary drugs are widely applied in animal husbandry to prevent and treat diseases. After consumption, part of these chemicals and their metabolites will be excreted and may eventually reach grasslands, for example when the animals are pastured or when their manure is sprayed over the land as a fertilizer. In peat-districts of the Netherlands, it has been observed that the farming system (organic or conventional) influences insect abundance in cowpats, with conventional management having less insects (Geiger et al., 2010).

In an effort to unravel the complex processes underlying the steady decline in meadow birds in the Netherlands, this study focuses on the potential role of agrochemicals and their impact on chick feed, i.e. insect populations, at cattle farms. To this purpose, we determined the presence of a broad spectrum of pesticides, biocides, some of their metabolites (i.e., those that are present in standard analyses packages) and antiparasitic medicines in Dutch cattle (predominantly dairy) farms that took part in meadow bird protection programs. We furthermore determined the presence of dung beetle populations in cow pats at those cattle farms that practiced grazing, also as an indicator for other arthropods. We explored the correlations between the detected chemicals and dung beetle occurrence.

2. Materials and methods

2.1. Farm selection

In the months June–August of 2018, we contacted 60 cattle farms in and around bird protection areas in the province of Gelderland in the Netherlands and asked if they would be willing to participate in a sampling campaign to determine the presence of a broad spectrum of pesticides, biocides and antiparasitic medicines at their farms. Except their willingness to participate in the project and their involvement in bird protection, other criteria for selection were their balanced geographical distribution over the whole province and the inclusion of sufficient organic/biodynamic farms for a comparison with conventional farms. Some farms were initially contacted by coordinators of meadow bird protection programs. Final agreement to participate in the study was established by telephone by the initiators of this study. Twenty-three cattle farms agreed: 8 organic and 15 conventional. All farmers answered a questionnaire (Table S1 in the Supplementary Material) consisting of 67 questions about their farm management; in particular about the use of external inputs like straw and concentrated feed that might contain pesticide residues. Concentrated feed in the Netherlands is produced from many different ingredients, like maize, soybean meal, wheat, barley, rye, palm oil, beet pulp, rapeseed meal, soybean oil, animal fats and citrus pulp (Nevedi, 2022). From the 23 farms, 22 participated in some sort of protection program for meadow birds, with measures like nest protection, delayed mowing, application of solid manure (instead of liquid manure; considered better for meadow birds), higher ground water tables and lower stocking rates. The one farm that did not participate in a protection program (farm 1 in Table S1) was an organic dairy farm that was included as a reference since it did not use any conventional inputs like chemical pesticides, medicines, straw and concentrated feed. The other 7 organic farms (Table S1) partly used conventional straw and medicines. The large majority of farms (21) were dairy farms with different side activities. One cattle farm produced meat cows (farm 18) and one raised heifers (farm 4). More details on the 23 selected farms are given in Tables S2 and S3 of the Supplementary Material.

2.2. Sampling and storage

On each farm, samples were taken from manure (solid and/or liquid), feed (concentrated feed, grass silage and/or hay) and/or soil (Table S2). Most farms used more than one type of concentrated feed for different categories of animals, like pregnant cows, calves among others. The type that was used most was sampled. In total, 76 samples were taken for pesticide analysis: 19 liquid cow manure samples, 7 solid cow manure samples, 24 samples of concentrated cow feed, 2 grass silage samples, 1 hay sample and 23 soil samples. Additionally, 23 extra manure samples were taken for analysis on anti-parasitic medicines (Table S2). Besides the 76 + 23 samples taken for chemical analysis, 18 samples from fresh manure pats were taken from the pastures with grazing cattle for the detection of dung beetles. All samples were taken in the period of 24 May–27 August 2018 with the exception of the two grass silage samples that were taken on October 9th and 30th, respectively. All sampling dates can be found in Table S2.

2.2.1. Soil

For soil sampling, we selected fields adequately reflecting the type of management at each farm, i.e. fields that were owned and managed by the farm for a long time. Soil samples were taken with an Edelman soil core sampler of 50 mm diameter and a sampling volume of approximately

300 cm^3. At each location, 10 samples were taken on two diagonals, i.e. 20 in total. Sampling depth was 0–20 cm. The 20 probes were manually mixed with a small galvanized metal spade in a galvanized metal bucket. From the mixture, a sample of approximately 1 kg was transferred to an Eijkelkamp plastic MeMopot® of 900 ml and stored at −18 °C until chemical analysis.

2.2.2. Feed and fodder

At each farm, a sample of the concentrated feed was taken; either from the sack with purchased feed, the automatic feed provider or directly from the silo. If multiple types of concentrated feed were used, preference was given to the dominant type of feed. Hay and silage samples consisted of at least 10 subsamples taken from different spots of the heap and at different depths. Concentrated feed and hay samples were kept in polyethylene bags of 3 l and stored at room temperature. Silage grass was kept in polyethylene bags in the freezer.

2.2.3. Manure

The dominant type of manure was sampled, either liquid or solid manure, or in a few cases both. Samples of liquid manure were taken from the manure pit with a rust-free metal bucket of 0.9 l on a 160 cm ash wood stick after careful mixing. In case of farms working with solid manure, at least 10 subsamples of solid manure from the cowshed were taken that were combined into a mixed sample. All samples were stored in MeMopots® of 900 ml at −18 °C until chemical analysis. For analysis of anti-parasitic medicines, samples were taken with the same sampling tool and put into 500 ml plastic jars.

2.2.4. Fresh manure pats

At 18 of the 23 participating farms (Table S2), the number of Coleoptera beetles was determined in one composite sample taken from ten randomly selected fresh manure pats in the field. The samples were taken with a metal spoon from 10 separate, relatively fresh cow pats during a visit between July 25th and September 21st 2018. These 10 different samples were combined into a plastic pot of 500 ml and stored at 5 °C for 1–59 days until the day of determination of the number of Coleoptera beetles, i.e. September 21st. The average weight of the composite samples was 394 g (±144 g).

2.3. Sample preparation and chemical analysis

2.3.1. Pesticides

Sample preparations and chemical analyses were performed by a commercial laboratory (Eurofins, Graauw, the Netherlands) from August 6th until 27th 2018, and consisted of the steps explained below.

2.3.1.1. Drying. Wet samples (i.e., manure and grass silage) were freeze-dried and their water content was determined based on the weight difference between the wet and freeze-dried samples.

2.3.1.2. Extraction. The freeze-dried and air-dry samples (i.e., concentrated feed, hay and soil) were milled and 1–10 g of each sample (depending on the matrix) was extracted with a mixture of three solvents: acetone (10 ml), petroleum-ether (10 ml) & dichloromethane (5 ml). Internal standards of PCB-153, triphenylphosphate and TDCPP were added to the dichloromethane as a control for the pre-treatment and the stability of the injection of the measuring device. This was tested before use. After addition of the solvents, 7 g of salt mixture was added to the extraction: sodium citrate (15.4%), sodium hydrogen citrate sesquihydrate (7.7%), magnesium sulphate (61.5%) and sodium chloride (15.4%). Extraction was performed during 2 min while being mixed at 640 RPM and subsequently the mixture was centrifugated for 10 min at 17,105*g*.

2.3.1.3. Clean-up. All samples were cleaned by thermal clean-up (in dry ice), followed by a chemical clean-up using florisil for dispersive solid-phase extraction (dSPE). For samples with a high pigment rate, i.e. manure, feed, fodder and hay, florisil was combined with graphitized carbon black (GCB) and primary-secondary amine (PSA).

2.3.1.4. Preparation for analysis. The solvents of the cleaned extract were evaporated at 50 °Celsius in a Zymark TurboVap LV Workstation. After reaching room temperature, the residue was dissolved in a 9:1 iso-octane/toluene mixture (*v*/v) for GC or in acidified methanol for LC. The residue was homogenized with a tube vortex and the extract was filtered through a round regenerated cellulose filter suitable for organic solvents.

2.3.1.5. Analysis. The supernatant of the centrifuged samples was analysed using GC–MS/MS (Agilent Intuvo 9000 GC coupled to an Agilent 7010B Triple Quad-Detector MS with Enhanced Masshunter operating system version B.07.06.2704 and MassHunter quantitative analysis software version 8.00, build 8.0.598.0). The GC was equipped with a G4513A injector and a G4520A autosampler. For LC-MS/MS, the Agilent 1290 series UHPLC was used with a TQ-5500 MS (Triple Quad-detector) of Sciex.

2.3.1.6. Quality control. After each series of 10 samples, a QC (Quality Control) sample was injected and analysed, containing all 664 substances (in a solvent without matrix) at concentrations matching the middle of the calibration line. Per group of 10 samples of the same matrix, spiked samples were added in order to determine the recovery of each substance. If the recovery was between 30%–80% or between 120 and 140%, the measured concentrations were corrected using the standard addition. In between 80 and 120% recovery, measured concentrations were not corrected.

2.3.1.7. Compounds, LOD, LOQ and confidence interval. Table S4 lists the 664 pesticide compounds analysed and their Limit of Quantification (LOQ); ranging from 0.1 microgram per kg for manure to 1 microgram per kg fresh weight for feed and soil. The 95% confidence interval of measurements above the LOQ is based on the maximal measurement uncertainty of 50% (in accordance with SANTE 11312/2021). This means that the real value is with a certainty of 95% between 0.5 and 1.5 times the measured value. The confidence interval has not been specified for measurements exceeding the Limit of Detection (LOD) but below the LOQ. These values were also included in the calculations.

2.3.1.8. Units. Concentrations in manure and grass silage were expressed on the basis of fresh weight as well as dry weight. Concentrations in concentrated feed and soil were expressed on the basis of the air-dry weight.

The analysed pesticides were classified as insecticide, fungicide, herbicide or biocide/repellent in accordance with the classification by the Dutch Board for the Authorisation of Plant Protection Products and Biocides (CtgB, 2021). Metabolites were put in the category of the parent substance. Caffeine was omitted, since it doesn't belong to one of the investigated classes.

2.3.2. Anti-parasitic medicines

The analysis of anti-parasitic residues was performed in conformance with EC guideline 2002/657/EC (EC, 2002). Immediately after defrosting, each manure sample was homogenized and subsequently two portions of 2 g were taken from each sample. To one of the two samples, the 21 anti-parasitic medicines (Table S5a) were added as a control (100 μg/kg). Then, 5 ml acetonitrile (ULC-MS grade; Actu-All, Oss, the Netherlands) was added and the samples were shaken. Extraction took place during 15 min (with head-over-head mixing) at a rotation frequency of 60 rpm with subsequent centrifugation at 3500 *g*. The supernatant was transferred onto dispersive solid phase extraction material containing 200 mg primary-secondary amine (PSA). The eluate was concentrated 25 times by evaporation of the eluate and reconstitution into a smaller volume, resulting in a 25-fold concentrated eluate. The extract was analysed using LC-MS/MS (Waters Acquity UPLC-AB Sciex Qtrap 6500) using the multiple reaction monitoring (MRM) mode. Each sample was analysed for 21 anti-parasitic drugs with insecticide properties, including 9 metabolites (Table S5a).

2.4. Coleoptera

The samples from fresh manure pats were weighted and mixed with water and led through a series of sequential metal sieves with a diameter of 20 cm and a mesh size of 4, 2 and 1 mm(s), respectively. The numbers of Coleoptera were counted by a Coleoptera specialist. The number of counted Coleoptera was expressed per kg fresh manure.

2.5. Relationship between estimated insecticide intake by cows and coleoptera in dung pats

For the 18 farms at which fresh manure pats were sampled, we explored the correlation between the pesticide concentrations in manure and the number of Coleoptera. The pesticide concentration in manure was quantified based on four scenarios:

a) The sum of all *pesticide* concentrations *measured* in manure from the manure pit;
b) The sum of all *insecticide* concentrations *measured* in manure from the manure pit;
c) The sum of all *pesticide* concentrations in fresh manure, *estimated* under the assumption that all pesticides consumed with dry feed (concentrated feed and hay) are being excreted;
d) The sum of all *insecticide* concentrations in fresh manure, *estimated* under the above assumption.

The distinction between pesticides and insecticides was made because herbicides typically dominate in concentrated feed, but do not specifically target insects such as Coleoptera. The distinction between measured and predicted manure concentrations was made because the concentrations measured in the manure pit may not accurately reflect the concentration in fresh manure pats, i.e. because of the degradation of pesticides during storage. The assumption that all pesticides consumed with dry feed are being excreted in manure is conservative, but not unrealistic. Data on excretion of orally consumed pesticides by cows and other animals are limited, but the available studies show that pesticides are either excreted largely unchanged (e.g., glyphosate in Japanese quail; Ruuskanen et al., 2020) or as metabolites (Croucher et al., 1985; Dauterman et al., 1959; Gaughan et al., 1978; Gutenmann et al., 1971; Robbins et al., 1957; O'Brien et al., 1961). Data on the toxic potential of metabolites relative to the parent compound (Bergmann et al., 2010) shows no clear pattern, and we therefore consider the assumption that metabolites are equally toxic as the parent compound defendable as a realistic worst-case.

The daily pesticide intake per cow was estimated by multiplying the average daily amount of concentrated feed and hay consumed per animal with the pesticide concentration measured in these respective media. The farmers were asked to estimate the amount of concentrated feed and hay consumed per cow per day. At most farms all cows got an identical amount, but at some farms neck collars determined individual amounts. In those cases, the average amount was used to calculate the average intake of insecticides per cow per day.

2.6. Potential environmental impacts

In order to determine the potential environmental impacts of the insecticides detected in different matrices, two assessments were performed for the terrestrial and aquatic environments:

- *Terrestrial*: comparison of the estimated pesticide load per ha, assuming polluted manure is used as a fertilizer, to the so-called LR50, i.e. the amount of active substance applied per ha at which 50% of the exposed test organisms die within 48 or 72 h.
- *Aquatic*: comparison of measured insecticide concentrations in manure to applicable surface water quality standards (valid in the Netherlands) in order to determine the manure dilution factor necessary to meet the water quality standards.

2.6.1. Assessment terrestrial impact

The insecticide load resulting from the application of polluted manure as a fertilizer was estimated per ha following two approaches:

1) multiplication of the typical manure application rate (30 tons of liquid manure per ha, containing 10% dry matter, or 12 tons of solid manure per ha, containing 25% dry matter) with the measured insecticide content of manure;
2) assuming that all insecticides consumed with concentrated feed and hay by the cows living at a particular farm are excreted unchanged in manure, and are subsequently distributed evenly over the farm land.

Most of the LR50 values used in the present study were taken from the International Union of Pure and Applied Chemistry (IUPAC, 2019).[1] The sources of all LR50 values are given in Table S6. LR50 values are typically derived from the survival rate of above ground living non-target arthropod organisms when exposed in the laboratory to the pure active ingredient after application, either to an inert substrate (e.g., glass) or a natural substrate (e.g., leaves or soil). Two test organisms are often used in an LR50 test, namely *Typhlodromus pyri* and *Aphidius colemani*, belonging to the predatory mites and insect families, respectively (EU, 2013; Grimm et al., 2001). These test organisms correspond better to the Coleoptera monitored in manure than the fishes and worms often used in LC50 tests. In addition, both test organisms represent important groups of arthropods that, like Coleoptera, are major food constituents of meadow bird chicks (Beintema et al., 1991).

2.6.2. Assessment aquatic impact

The applicable surface water quality standard (Annual Average Environmental Quality Standard, or AA-EQS) in the Netherlands, expressed in micrograms per litre, was taken from www.bestrijdingsmiddelenatlas.nl. The pesticide concentrations in manure (Table S6) were standardized to manure of 18.03% dry matter (i.e., the average dry matter content of manure from all 23 farms; standard deviation = 11.85%). The resulting concentration in micrograms per kg manure was converted to micrograms per litre by multiplication with a factor of 1.1 (i.e., the specific density of manure with a dry matter content of 18.03%).

2.7. Statistical processing

The correlation between the calculated total daily insecticide intake by cows and the number of Coleoptera found in fresh manure was tested by the Kendall test (IBM SPSS, Version 25). The Mann-Whitney Wilcoxon *U* test was used to test whether the measured values of two groups were significantly different. The standard deviation of specific averages is shown in order to express their variability.

3. Results

3.1. Pesticides, biocides and their metabolites

In total, 129 different pesticides, biocides and metabolites were detected in the 76 samples in concentrations above the LOD. Fig. 1 shows the total number of substances found per matrix. In manure, many more substances were found than in soil and concentrated feed. The lowest number of substances was found in soil. An overview of all substances detected in the 76 samples can be found in Table S6, together with their LOQ, LOD and the dry matter content of the manure samples.

Table 1 shows the distribution of the detected pesticides, biocides and metabolites over the different classes of pesticides, i.e. herbicides, fungicides, insecticides, biocides & repellents. Manure contains the highest number of fungicides, herbicides and insecticides. The number of biocides & repellents is relatively low in all matrices. A relatively large number of insecticides (i.e., 13) was found in the 3 fodder samples (i.e., hay and silage).

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[1] The LR50 value can be found in the IUPAC database by selecting the substance and scrolling to the section 'Ecotoxicology'.

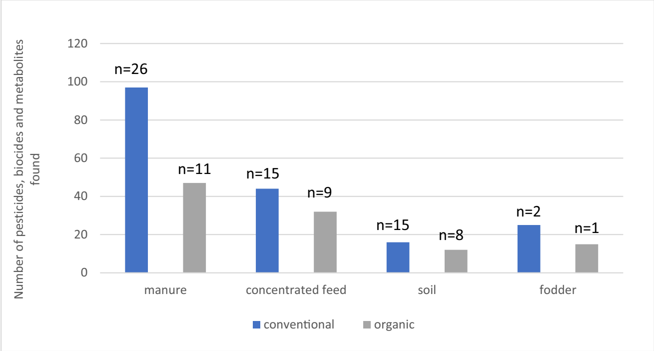

Fig. 1. The number of different pesticides (*including the insecticide synergist piperonyl butoxide*), biocides and metabolites found at 15 conventional and 8 organic cattle farms in four matrices (*n represents the number of samples*).

Table 1
Distribution of the detected pesticides, biocides and metabolites over the different classes of pesticides, i.e. fungicides, herbicides, insecticides and biocides & repellents[a,b,c].

Matrix	Fungicides	Herbicides	Insecticides	Biocides & repellents
Concentrated feed (n = 24)	19	14	17	3
Fodder (n = 3)	7	7	13	3
Manure (n = 26)	42	25	37	3
Soil (n = 23)	7	6	4	1

[a] Metabolites were put into the category of their parent compound.

[b] Classification according to the Dutch Board for the Authorisation of Plant Protection Products and Biocides (Ctgb, 2021).

[c] n represents the number of samples.

Insecticides were found in 92% of the concentrated feed samples, 88% of the manure samples and 17% of the soil samples. The most frequently detected insecticides in concentrated feed were chlorpyriphos-methyl (46%), pirimiphos-methyl (58%), cypermethrin (67%) and the synergist piperonyl butoxide (71%). From these four insecticides, three were also detected in manure: pirimiphos-methyl (4%), cypermethrin (12%) and piperonyl butoxide (71%). Diphenylamine (12%) and thiamethoxam (17%) are examples of insecticides detected in manure but not in concentrated feed. In addition, 32 other insecticides (including metabolites) were detected in manure, the majority of which appeared only in a few samples (Table S6). The metabolite of DDT, p,p-DDE, was detected in 13% of the soil samples and DEET in 4%.

Fungicides were detected in 100% of the 24 samples of concentrated feed, in all 3 fodder samples, in all 23 soil samples and in 73% of the 26 manure samples. Herbicides were detected in all 3 samples of fodder and in 100% of the 23 soil samples. Herbicides were detected in 92% of the concentrated feed samples and also in 77% of the 26 manure samples. AMPA, a metabolite of glyphosate, was detected in 100% of the soil samples, glyphosate in 17% and biphenyl in 96%. The repellent anthraquinone was found in 78% of the soil samples (Table S6).

Table 2 lists the summed average concentrations of the substances over the different classes of pesticides, i.e. fungicides, herbicides, insecticides and biocides & repellents, and summed over all classes. It shows that the concentrations detected in soil are relatively modest when compared to the other matrices. Relatively high concentrations of herbicides were detected in concentrated feed and manure. Relatively high concentrations of insecticides were found in fodder, followed by manure and concentrated feed. The average concentrations of herbicides and insecticides detected in manure are comparable to those in concentrated feed, but the average concentration of fungicides and biocides/repellents is much higher in manure. The relatively high standard deviations are indicative of substantial variation between the farms.

The major part of the average amount of herbicides detected in concentrated feed and manure consisted of glyphosate and its metabolite AMPA. In concentrated feed, glyphosate contributed 76% to the total herbicide concentration and its metabolite AMPA 22%. The other 13 herbicides contributed on average only 2% to the total herbicide concentration in concentrated feed.

The average total pesticide concentration in concentrated cattle feed of conventional farms (971.6 μg/kg) was 3.7 times higher than in that of organic farms (261.2 μg/kg; $p < 0.01$; two-tailed Mann-Whitney Wilcoxon test). In soils, the average total pesticide concentration at conventional cattle farms was a factor of 2.53 higher than at organic cattle farms (74.22 μg/kg vs. 29.30 μg/kg), but this difference was not significant ($p > 0.05$; two tailed Mann-Whitney Wilcoxon test). The difference between conventional and organic cattle manure was also not significant. The average total pesticide concentration in the dry matter of manure was 1.74 times higher at conventional farms (1129 μg/kg dwt) than at organic farms (647 μg/kg dwt; $p > 0.05$; two tailed Mann-Whitney Wilcoxon test). All mentioned concentrations can be found in Table S6, sheet 2.

3.2. Top 8 insecticides in concentrated cattle feed

Table 3 lists the average and highest concentrations of the top 8 insecticides detected in concentrated cattle feed (i.e., cypermethrin, pirimiphos-methyl, chlorpyrifos-methyl, chlorpyrifos-ethyl and piperonyl-butoxide; together responsible for 93.4% of the average insecticide load in concentrated feed), including their detection frequency. The average has been calculated over those samples in which the substance was detected. The table shows that pirimiphos-methyl had the highest average concentration (57 μg/kg) as well as the highest maximum concentration (270 μg/kg). The average summed concentration of all insecticides in concentrated cattle feed samples at organic farms was around 31 times lower (6.21 μg/kg) than in concentrated cattle feed at conventional farms (192.8 μg/kg; $p < 0.01$; two-tailed Mann-Whitney Wilcoxon test). Fig. S1 lists the incidence of all insecticides in concentrated cattle feed. The original measured data can be found in Table S6, sheet 2. In Table S6 sheet 4 the total content of insecticides in dry feed is specified which was used to calculate the total daily uptake of insecticides by cows (Table S8).

Table 2
Summed average concentrations (in micrograms per kg dry matter) over each pesticide class and the summed total average concentration in different matrices of 23 cattle farms (standard deviation between brackets).

Matrix	Fungicides	Herbicides	Insecticides	Biocides & repellents	Sum
Concentrated feed[a] (n = 24)	43.7 (38.4)	499.1 (576.5)	102.7 (198.6)	9.4 (32.5)	654.9 (666.4)
Fodder[b] (n = 3)	39.4 (17.9)	18.1 (8.2)	335.1 (436.2)	35.3 (52.1)	427.9 (473.3)
Manure[b] (n = 26)	259.0 (623.9)	404.3 (511.8)	175.3 (515.9)	96.1 (327.8)	934.7 (1241.8)
Soil[a] (n = 23)	3.9 (2.7)	47.2 (76.1)	2.3 (8.4)	9.5 (26.5)	62.9 (77.1)

[a] Per kg air-dry weight.

[b] Per kg dry weight.

Fig. 2. Fresh cattle manure without holes produced by cows that feed on hay with permethrin and bifenthrin at conventional farm 18 (left), fresh manure at organic farm 1 directly colonized by Coleoptera (*Sphaeridium scarabaeoides*; middle), and manure drawn open by birds (lapwings) in search for insect food at farm 1 (right).

3.3. Anti-parasitic medicines found in manure

Only on four cattle farms anti-parasitic medicines were found in manure, namely farms 8, 9, 15 and 18 (Table S7). The concentrations in the liquid manure were relatively low and close to the LOQ, except for farm 18 where 12 micrograms of ivermectine was reported as well as 8 other anti-parasitic medicines at low concentrations.

3.4. Coleoptera in fresh cattle manure

The extent of colonization of fresh manure by Coleoptera was remarkably different at first sight; caused by the fact that some Coleoptera create well-visible entrance holes in the manure surface (Fig. 2). A total of 50 adult Coleoptera beetles and one beetle pupa, but no larvae, were detected in the cow pat samples from the 18 farms, belonging to 14 species from 8 families (Table S9). The number of Coleoptera per kg of manure at organic farms (6.5 ± 7.7) did not differ significantly from that at conventional farms (7.8 ± 17.8; $p > 0.05$; two tailed Mann-Whitney Wilcoxon test).

The numbers of living Coleoptera did not correlate significantly with the summed concentrations of measured pesticides in manure from the manure pit/cowshed (Kendall $\tau = 0.166$, $p = 0.360$), nor with the summed concentrations of measured insecticides (Kendall $\tau = 0.168$, $p = 0.359$). However, a significant negative correlation was found between the numbers of living Coleoptera and the average estimated daily uptake of insecticides per cow, as given in Table S8 (Kendall $\tau = -0.361$; $p = 0.05$). Fig. 3 shows that the number of Coleoptera was very low at farms exceeding an estimated daily insecticide uptake of 250 µg per cow. The correlation between the total uptake of pesticides per day per cow and the number of Coleoptera was almost significant (Kendall $\tau = -0.351$, $p = 0.056$). All correlation tests conducted are indicated in Fig. S2.

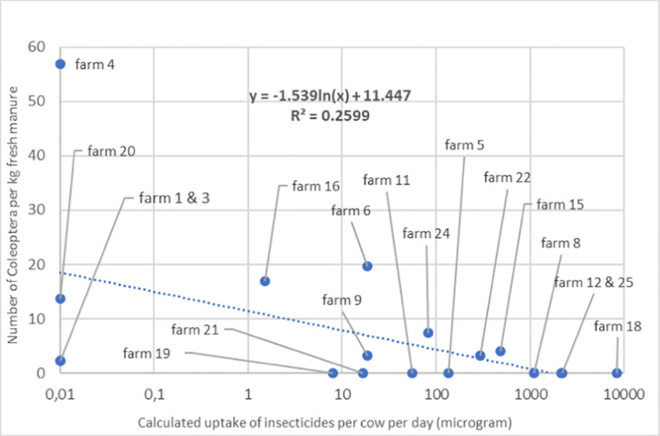

Fig. 3. Number of Coleoptera per kg fresh manure plotted against the estimated uptake of insecticides with dry feed (concentrated feed or hay) per cow per day (µg per cow per day) for the 18 cattle farms that pastured their cattle.

3.5. Pesticide loads and LR50 values

Table 4 shows the estimated pesticide loads per ha if polluted manure is applied as a fertilizer, following the two approaches outlined in the Methods section (i.e. based on measured manure concentrations, and based on the assumption that the entire amount of a pesticide consumed with concentrated feed is excreted unchanged and applied to the land). Table 4 only includes pesticides for which an LR50 value was available and was exceeded at one or more farms. Method 1 resulted in the LR50 of one or more pesticides being exceeded at 7 farms, i.e. farms 3, 6, 8, 9, 17, 23, & 25. Three of these farms were organic (Table S2). Method 2 resulted in the LR50 of one or more pesticides being exceeded at 14 farms; all conventional (farm 4,5,6,8,9,10, 12,15,17,18,20,22,23 & 25). The detailed data and calculations per farm can be found in Table S6, sheet 3 (method 1) & sheet 5 (method 2).

3.6. Potential environmental impact on the aquatic environment

Table 5 compares the measured insecticide concentrations in manure to the applicable water quality standards, i.e. the AA-EQS. This is the annual average concentration that should not be exceeded in order to protect the aquatic ecosystem and human health. The insecticide concentration in manure exceeded the AA-EQS by a factor of 232 for imidacloprid to 3.1 million for deltamethrin.

Except these insecticides, there were 45 other substances (fungicides, herbicides, one repellent and other insecticides) exceeding the aquatic AA-EQS in manure (Table S6, first worksheet, column CU). These will not be further discussed since the focus is on insecticides.

4. Discussion

We analysed the presence of 664 pesticides (including biocides and some metabolites) and 21 anti-parasitic medicines in four different matrices (concentrated feed, fodder, manure and soil) at 8 organic and 15

Table 3

Average of positive measurements and highest maximum concentrations of the 8 insecticides with the highest detection frequency in concentrated cattle feed (µg/kg air-dry weight; average taken over samples tested positively; standard deviation between brackets).

Insecticide/synergist	Average (µg/kg)	Highest (µg/kg)	Detection frequency (n = 24)
Piperonyl-butoxide	51.9 (75.1)	265	71%
Cypermethrin	30 (62.3)	247	67%
Pirimiphos-methyl	57 (81.1)	270	58%
Chlorpyrifos-methyl	29 (17.1)	102	46%
Bifenthrin	2 (1.5)	5	21%
Chlorpyrifos-ethyl	3 (1.1)	4	17%
Deltamethrin	1 (0.8)	2	8%
Fenazaquin	2 (0.6)	3	8%

Table 4
Estimated pesticide loads if polluted manure is applied as a fertilizer and subsequent exceedance of LR50 values.

Pesticide	LR50 (gram/ha)	Method 1[a]			Method 2[b]		
		Load[c] (gram/ha)	# farms >LR50	% farms >LR50	Load[c] (gram/ha)	# farms >LR50	% farms >LR50
Cypermethrin	0.0029	0.010 (0.051)	2	8.7	0.065 (0.185)	13	56.5
Bifenthrin	0.113	Not detected	0	0	0.006 (0.024)	1	4.3
Chlorpyriphos-ethyl	0.2	Not detected	0	0	0.022 (0.072)	1	4.3
Deltamethrin	0.00439	0.016 (0.052)	4	17.4	0.0004 (0.002)	1	4.3
Imidacloprid	0.022	0.001 (0.006)	1	4.3	0.0002 (0.001)	0	0
Lambda-cyhalothrin	0.0037	0.003 (0.015)	2	8.7	0.00003 (1.25×10^{-4})	0	0
Spirodiclofen	2.4	0.284 (1.452)	1	4.3	0.001 (0.005)	1	4.3
Total number of farms	–	–	7	21.7	–	14	60.8

[a] Pesticide load estimated based on measured manure concentration and application rate of 3 tons dry matter from manure per ha;

[b] Pesticide load estimation based on the assumption that the entire amount of a pesticide consumed with dry feed by all cows living at a farm is excreted (unchanged, or as pesticidal active metabolites) and applied to the land;

[c] Average pesticide load over all 23 farms (standard deviation between brackets); assuming zero concentration if value was below LOD.

conventional cattle farms. We found a negative correlation between the number of Coleoptera in fresh dung pats and the estimated amount of insecticides consumed by cows. We furthermore showed that various ecological standards may be exceeded if the polluted manure is distributed over land as a fertilizer, or if it reaches surface water in diluted form. In this discussion section, we reflect on our methods and the implications of our results.

4.1. Pesticides at dairy farms

Pesticides and other agrochemicals are used on a regular basis in agriculture. Examples include the application of herbicides to grasslands, insecticides to feed crops, fungicides to soils and biocides in cow sheds. As such, it does not come as a surprise that we detected a wide range of different pesticides in the concentrated feed, fodder, manure and soils of the dairy farms. Although many other studies have analysed pesticides in agricultural systems, very few studies specifically focus on the route from the feed, via manure, to the land, and the potential adverse impacts on the field entomofauna. Studies on pesticides in feed products (e.g. Nag and Raikwar, 2011; Bedi et al., 2018) generally focus on safety of the resulting products (e.g. the meat or the milk) for human consumption, or on the well-being of the animals. Studies on manure generally focus on veterinary medicines and biocides, ignoring the potential role of the wider group of pesticides, and especially insecticides. We identified only two studies in the published literature that simultaneously measured pesticides in feed and manure (Zhao et al., 2013; Muola et al., 2021), but they only considered a limited number of pesticides. Zhao et al. (2013) determined the residual levels of 8 organochlorine pesticides (OCPs) in feed and cow manure and found a comparable range in manure and feed (based on dry matter). Muola et al. (2021) only conducted glyphosate measurements in chicken feed and manure, which are hard to compare with cattle feed and manure.

Table 5
Measured concentrations of insecticides from previous table in manure and comparison with applicable water quality standards (AA-EQS) in the Netherlands for those manure samples in which the substances were found (ND = Not Detected).

Pesticide	Average concentration in manure (μg L^{-1})[a]	Aquatic AA-EQS (μg L^{-1})	Ratio[b]
Cypermethrin	5.9	8×10^{-5}	73,245
Bifenthrin	Not detected	1×10^{-3}	–
Chlorpyriphos-ethyl	Not detected	3×10^{-2}	–
Deltamethrin	9.3	3.1×10^{-6}	3,000,678
Imidacloprid	2.1	8.3×10^{-3}	255
Lambda-cyhalothrin	3.1	2×10^{-5}	154,595
Spirodiclofen	489.4	2.5×10^{-2}	19,574

[a] On basis of the calculated average dry matter content of 18.03% and on basis of specific weight of 1.1 kg per litre manure.

[b] The calculated ratio of all substances can be found in Table S6, sheet2, column CV.

The total number of pesticides and the total amounts detected in our study were generally highest in manure, followed by concentrated feed, fodder and soil (Fig. 1 and Table 2). This seems to make sense from a mechanistic perspective if one assumes that most pesticides will not be absorbed and metabolized by the cattle. In that case, digestion of feed in the gastrointestinal tract will concentrate the pesticides, ultimately resulting in higher concentrations in manure. However, other explanations for the relatively high pesticide concentrations in manure are also possible, such as the presence of other exposure routes for cattle not addressed in our study. Examples include the application of pesticides as veterinary medicines (e.g., deltamethrin, which is often used as an antiparasitic drug) or inhalation and dermal exposure after disinfection of the accommodation and the use of non-organic straw for bedding of animals. Furthermore, it should be kept in mind that some pesticides may be absorbed and metabolized by the cows. This may explain why some pesticides, like the synergist piperonylbutoxide, were consistently detected in concentrated feed and manure, whereas other pesticides, like many of the pyrethroids, were detected in concentrated feed only. In the latter case, these compounds may have been transformed into metabolites that were not analysed in our project. Well-known metabolites of cypermethrin, for example, include trans-DCVA,[2] cis-DCVA and 3-PBA (EFSA, 2018).

Considering the different pesticide classes present in feed (Tables 1 & 2), it stands out that herbicides dominate quantitatively in concentrated feed whereas insecticides dominate in fodder. The first result is in line with the relatively high use of herbicides on feed crops, in combination with the concentrated nature of the feed. Typically, application rates of insecticides on feed crops are much lower than those of herbicides. For example, the recommended dose of the most widely used herbicide, glyphosate (often marketed under the name ‘Roundup’), ranges between 800 and 2400 g per ha, while typically less than 100 g per ha is used for insecticides (Ctgb, 2021). Data on pesticides in concentrated feed are very scattered and not easy to find, but our results are generally in line with the limited sources available in public literature (e.g. Mol et al., 2014). The relatively high insecticide levels in fodder, particularly of propoxur and permethrin, could be explained by the use of these compounds to disinfect the

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[2] Also abbreviated as trans-DCCA and cis-DCCA.

accommodation, or during storage. Permethrin is marketed as a disinfection agent under the name Permas-D, whereas propoxur is used to treat pets against fleas and ticks (CBG, 2019). However, the origin of these compounds remains rather speculative. As we analysed only a limited number of fodder samples, a more extensive sampling campaign is needed to determine whether our findings are representative.

4.2. Coleoptera

The number of living Coleoptera in cow pats varied between 0 and 56.9 per kg manure (Table S9). Comparison with other studies is difficult since most studies report the number of insects using different experimental designs (e.g. Lee and Wall, 2006; Geiger et al., 2010). Lee and Wall (2006) did not establish the Coleoptera numbers in naturally deposited fresh manure, but constructed artificial cow pats, in which, after a certain period, larvae were counted. Geiger et al. (2010) counted adult Coleoptera, but expressed the numbers per cow pat. Due to those differences, their results cannot be compared directly with the counts in our study. Nevertheless, it is clear that the total number of adult Coleoptera in the study of Geiger et al. (2010) was in the same range as in our study (21.11–35.67 per cow pat), assuming the average cow pat weighed more than 1 kg. Part of the observed variation in Coleoptera numbers may be explained by differences in the age of the cow pats, as fresh pats tend to have higher numbers of adult Coleoptera than slightly older pats (Lee and Wall, 2006). However, we think this impact was limited since we specifically sampled fresh cow pats without any crust (that is formed within hours under hot conditions like in the summer of 2018). We therefore hypothesize that the variation can, at least partly, be attributed to the presence of insecticides in manure, originating from the dry feed (concentrated feed and hay). This is supported by the correlation we found between the estimated insecticide intake with dry feed and the number of Coleoptera in cow pats. The fact that this correlation was not found for measured insecticide concentrations in manure from the manure storage might be explained by the fact that the manure from the manure storage is likely to have a different composition, due to chemical conversions that take place during storage and due to other feed that cows get in the winter period. The presence of active metabolites of many insecticides is likely to remain undetected, since most of such metabolites were not measured. The correlation between insecticides in dry feed and Coleoptera counts is supported by observations at individual farms, e.g. the coincidence of high levels of permethrin and bifenthrin in the hay of farm 18 with the absence of Coleoptera, and the high number of Coleoptera at farm 4 coinciding with an estimated zero intake of insecticides because the cows did not consume any dry feed during that period. There were also several farms where no Coleoptera were found, even though the calculated insecticide intake was low, e.g. farms 1, 3, 19 and 21. One potential explanation might be that the fodder at these farms contained significant amounts of insecticides which we did not include in our analyses.

Next to insecticides, Coleoptera numbers can be impacted by other factors such as landscape structure (Roslin and Koivunen, 2001) and the presence of anti-parasitic medicine residues (Wardhaugh, 2005; Tixier et al., 2016). We think landscape structure played a minor role in our study since all 18 livestock farms were located in similar landscapes dominated by animal husbandry. Anti-parasitic medicines were detected in the manure of four farms, i.e., 8, 9, 15 and 18. It is possible that these substances contributed to the toxic nature of the manure, but we think their impact was generally limited because of two main reasons. First, concentrations were generally low, with the exception of farm 18. However, the absence of Coleoptera at farm 18 can also be attributed, at least partially, to the very high quantities of permethrin detected in hay and manure (>500 μg/kg dry matter). Second, we sampled the manure storage of dairy farms for anti-parasitic medicines. It contained manure obtained predominantly in spring time when the application levels are typically high. The cow pats in the pastures were sampled for Coleoptera in the summer months, i.e. from July 25th to September 21st 2018. It is likely that, by that time, the cows already had excreted most of the anti-parasitic medicines they were treated with during springtime.

4.3. Differences between organic and conventional farms

As expected, higher levels of pesticides were generally encountered in the concentrated feed, fodder, manure and soils of conventional farms than of organic farms, but the differences were smaller than might be expected and, in most cases, not significant. The difference was significant for concentrated feed only. The difference is in line with other studies that compared the presence of pesticides between conventional and organic farming (Baker et al., 2002; Witczak and Abdel-Gawad, 2012; Geissen et al., 2021). Although the use of synthetic pesticides is forbidden at organic farms, their presence can have different explanations, e.g. the allowed use of non-organic inputs (like straw and anti-parasitic medicines), the legacy of persistent pesticides from the pre-organic era, the diffuse blanket of pesticides present in the environment or a questionable origin of (part of) the organic feed. The general presence of cypermethrin and pirimiphos-methyl is likely caused by disinfection during storage and transportation. Regulations for organic trade do not regulate disinfection of transport vehicles, like ships and trucks (EU, 2018). The lower levels of pesticides in concentrated feed at organic farms did not translate into significantly higher numbers of Coleoptera in cow pats. A potential explanation is the fact that even the relative low insecticide content of organic concentrated feed (at 6.21 μg per kg dry matter) was too high for good Coleoptera colonization of manure. Additional reasons might be the relative low number of farms at which cow pats were sampled for Coleoptera in combination with the high natural variation in Coleoptera numbers.

4.4. Environmental risk assessment of polluted manure

The potential ecological impacts of pesticides transferred to the environment through the application of polluted manure are rarely considered in regulatory and scientific assessments, and the few studies that do, only consider a very limited number of pesticides (e.g., Muola et al., 2021). However, it is known that carryover of herbicides via manure can damage vegetable and flower crops, as previously reported by the UK Health Safety Executive for farm yard manure containing aminopyralid residues (https://www.hse.gov.uk/). Comparable problems have been reported for the herbicide clopyralid in cattle manure compost (Watanabe et al., 2019).

In the present paper, we explore different ways to assess the potential environmental impacts of pesticides contained in manure. One option is to calculate the total pesticide load applied with manure, and compare this to the load typically applied directly. In our case, the average load applied with manure equals 4.29 g per ha, assuming a yearly application rate of 3 tons of dry matter with (either liquid or solid) manure per hectare. This is a factor of 1305 lower than the average of 5.6 kg of active ingredient per ha yearly applied in arable farming in the Netherlands (CBS, 2016). Considered in this context, the application rate of pesticides with cattle manure can be considered low. However, it should be kept in mind that only 20–25% of the active ingredients allowed on the European market were included in our non-target analysis and that pesticide metabolites were largely ignored. Examples include important metabolites of cypermethrin, such as the chlorinated trans-DCVA,[3] cis-DCVA and 3-PBA (EFSA, 2018), and pirimiphos-methyl metabolites, containing the pyrimidine dialkyl phosphorothioate structures (EFSA, 2005). Ecotoxicological data for such metabolites are typically lacking, although bioassays on worms indicated that the DCVA metabolite was significantly more toxic than the mother compound cypermethrin (Guojun et al., 2015).

Another option to assess the potential ecological impacts of the pesticides contained in manure when used as a fertilizer, is to compare the application rate of the individual pesticides to the LR50. When this is done at the individual farm level, the results (Table 4) indicate that the LR50 values of cypermethrin, chlorpyriphos-ethyl, bifenthrin, deltamethrin, imidacloprid, lambda-cyhalothrin and spirodiclofen could be exceeded at 7 or 14 farms,

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[3] Also abbreviated as trans-DCCA and cis-DCCA.

respectively, depending on how the pesticide load per ha is being calculated. It can be debated whether these calculations provide a realistic indication of the potential ecological impacts of the insecticides, since LR50 values are based on direct exposure to the active ingredient whereas the pesticides in manure are likely to be partly absorbed to organic matter. Nonetheless, we consider the LR50 value to provide a more realistic indication of ecological effects than most other standards since it captures the short-term toxicity (48–72 h) to terrestrial arthropods directly after application, whereas other standards typically relate to less relevant species, exposure routes and exposure times.

We did not consider the long-term impacts of the insecticides. Tennekes (2010) has shown that the toxic effect of chemicals can accumulate over time in case those chemicals show irreversible receptor binding. Sánchez-Bayo (2009) demonstrated that short-term exposure (0.9 days) of *Cypridopsis vidua* to an undiluted imidacloprid solution triggers the same lethal effects as 5.2 days of exposure to a solution that is diluted by a factor of 1000. Examples of pesticides found in this study with time dependent expression include fipronil, permethrin, imidacloprid (Tennekes and Sanchez Bayo, 2013) and boscalid (Simon-Delso et al., 2018). It can be debated whether cypermethrin should also be included in this list since it has identical metabolites as permethrin, namely the trans-DCVA, cis-DCVA and 3-PBA (EPA, 2004), and part of its toxicity likely is attributable to these metabolites. In addition to irreversible receptor binding, the risk assessment does not include potential cocktail effects of the (3–45) pesticides found in manure (Backhaus et al., 2013). Furthermore, the effect of co-formulants that producers of pesticides add to their formulations and that are intended to enhance the toxic impact on living organisms, was not taken into consideration (Backhaus et al., 2013). As a result, our ecotoxicological assessment of the effects of pesticides might still be too conservative.

As a final indicator of potential ecological effects, we compared the insecticide levels detected in manure to the applicable water quality standards. Pesticides contained in manure can reach surface waters, for example if some the manure is incidentally sprayed over bordering ditches or through runoff. Table 5 indicates that the manure of positively tested samples must be diluted by up to a factor of 3 million to meet the applicable water quality standard for deltamethrin. This effectively means that 16.7 ml of manure is sufficient to pollute a ditch (holding 50 cm of water; 1 m wide and 100 m long) to the level of the aquatic AA-EQS of deltamethrin. Again, it can be argued that not all the deltamethrin contained in manure will be bioavailable, but many of the organic material in manure may be available as dissolved organic matter.

4.5. Relation of findings with meadow bird populations

Although in this study only Coleoptera counts were conducted, it is likely that the insecticides detected will have similar effects on many other invertebrate taxa. Faecal analysis has shown that almost all major invertebrate taxa found in grasslands, from 1.5 mm aphids to beetles and craneflies ≥15 mm, occur in the diet of chicks of black tailed godwits, lapwings and other meadow birds (Beintema et al., 1991). We therefore argue that the application of polluted cattle manure from dairy farms should be further explored as a potential cause for the decline of meadow birds in the Netherlands and elsewhere where cattle manure is produced and applied to grasslands.

5. Conclusions

A total number of 129 pesticides was detected in 76 samples of manure, feed, fodder and soil of conventional and organic dairy farms above the LOD. The largest class of pesticides encountered in manure was the fungicides (42), closely followed by insecticides (37) and herbicides (25; including metabolites). The average concentration of insecticides in organic concentrated cattle feed was 31 times lower (6.21 μg/kg), than in conventional concentrated cattle feed (192.8 μg/kg), whereas the total pesticide content was 3.7 times lower. The number of Coleoptera in cow pats showed a significant negative correlation with the estimated insecticide uptake by cows. Exploratory assessments based on LR50 values indicate that the carryover of insecticides from feed and other sources to manure, and ultimately to the field, is a potential reason of concern for the entomofauna in the field. We hypothesize that this carryover may explain part of the decline in meadow birds observed in the Netherlands and conclude that continued research is required to improve our understanding of the interactions between pesticides, insects and meadow birds. Comparison of pesticide concentrations in manure with the aquatic AA-EQS valid in the Netherlands also indicated that even tiny manure emissions to surface water may have a severe ecological impact.

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CRediT authorship contribution statement

Jelmer Buijs: conceptualization, investigation, supervision, data curation, writing-original draft, project administration, funding acquisition. Ad Ragas: validation, methodology, writing-review & editing. Margriet Mantingh: conceptualization, methodology, investigation & data curation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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