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Residues of pesticides in dairy cow rations and fly treatments reduce the number of Coleoptera in dung

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ABSTRACT

There is a global pattern of a decline in insect abundance and diversity. The presence of residues of pesticides in animal feed and fly treatments results in polluted animal dung, which may hamper the survival of insects feeding and breeding in and on dung. To study the route from pesticide contamination in the feed and the dung to insect survival in dung pats, two experiments were performed. In Experiment 1, the feed and dung of dairy cows were collected on eight Dutch dairy farms (six conventional, two organic). All the feeds in the ration as well as the dung were analysed for the presence of pesticides. In Experiment 2, dung was collected from a herd of dairy cows and was spiked with four concentrations of deltamethrin. In both experiments, a field experiment was implemented with artificial dung pats of 2 kg of fresh dung. The dung pats were retrieved after 7 and 14 days (Experiment 1), or 7, 14, 21, and 35 days (Experiment 2), and invertebrates in the dung were counted. A total of 70 pesticides were detected in the different types of feed of the eight farms. The concentration of pesticides in the dung was on average 423 μg kg^{-1} DM (range of 112–1980 μg kg^{-1} DM). In Experiment 1, the number of Coleoptera present in dung was negatively correlated with the pesticide concentration, and more Coleoptera were present in the dung of organic farms (23.7 on organic farms vs. 11.4 Coleoptera on conventional farms, per 500 g freshly deposited dung). In Experiment 2, the dung which was not spiked with deltamethrin contained more than twice as many Coleoptera larvae and adults (5.6 Coleoptera per 500 g freshly deposited dung on treatment without deltamethrin vs. 2.2 with highest concentration of deltamethrin). We conclude that pesticides are widely present in feed for dairy cows and that a number of these pesticides are transferred to dung. The pesticides have a negative effect on the number of Coleoptera and as a consequence may also affect ecosystem services such as the disappearance of dung pats and the presence of insects in dung pats as feed for farm birds or bats.

1. Introduction

Data suggest a global pattern of decline in insect abundance and diversity (e.g. Benton et al., 2002; Woodcock et al., 2015; Cellabos et al., 2017, Hallmann et al., 2017; Barmentlo et al., 2021). As insects play a crucial role in the functioning of ecosystems (Beynon et al., 2012, 2015; Gittings et al., 1994; Kim, 1993), this decline may affect ecosystem services such as pollination, nutrient cycling and pest control (Beynon et al., 2015; Kim, 1993; Klein et al., 2003; Tschnarke et al., 2005); in addition, it may affect organisms that feed on insects, as insects are an important part of the food web and a food source for many living organisms, such as meadow birds on grasslands (Wilson et al., 1999; Vickery et al., 2001) and bats that forage near dairy cows (Catto et al., 1996; Ancillotto et al., 2017). The global decline in insects may have several causes, e.g. habitat loss due to deforestation, climate change (Gilles, 2019) and agricultural intensification (Wilson et al., 1999; Hutton and Giller, 2003; Gilles, 2019), including the use of insecticides and/or other pesticides (Hempel et al., 2006; Boxall et al., 2007; Brühl et al., 2021).

Different types of pesticides may directly or indirectly affect the variety of insects. The most common ways in which they affect insects is directly through the use of the agents on agricultural fields or indirectly through the drift of the agents from neighbouring fields (Ochoa and Maestroni, 2018). However, recent research has shown that insecticides and/or other pesticides are also present in animal manure (Wohde et al., 2016; Buijs and Samwel-Mantingh, 2019), which could affect insects who live and forage on dung. The sources of the pesticides in dung are often not clear (Buijs and Samwel-Mantingh, 2019). The most likely

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sources are (i) the use of veterinary medicine and anti-parasite agents against ticks, flies or helminths and (ii) the presence of pesticides in animals feed.

In the case of veterinary medicine and anti-parasite agents, many farmers use pour-on applications against flies and parasites, and such applications often contain macrocyclic lactones (ivermectin, eprinomectin) or pyrethroids (deltamethrin, cypermethrin). Macrocyclic lactones migrate through the skin and a major part is excreted in the dung (Tixier et al., 2016; Venant et al., 1990; Virlouvet et al., 2006), causing a strong negative effect on the presence of flies and beetles (Wardhaugh et al., 1998; Floate et al., 2015; Vale et al., 2004; Virlouvet et al., 2006) as well as entailing other environmental risks (Stowa, 2019). Negative effects on the presence of beetles have even been observed with values below detection limits (Wardhaugh et al., 1998). The wide use of medicine against parasites may thus lead to concentrations in the dung at which the dung fauna will not survive and/or the breeding of insects is inhibited.

With regard to pesticides in animal feed as a source of pesticides in dung, it has been observed that some animal feed (concentrates as well as grains and by-products) may contain pesticides. Walorczyk (2008) found 15 chemical substances in 145 animal feed samples, and Walorczyk and Drożdżyński (2012) found residues of pesticides in 17% of 900 feed samples. The pesticides present in animal feed will be consumed and at least partly excreted in dung or urine, depending on the type of pesticide, and they could have a negative effect on the dung as feeding and breeding habitat for insects. However, the presence of pesticides in animal feed is usually not well documented or published. This applies even more to roughages than to concentrates. A quantification of the pesticides present in the dung of dairy cows with different diets and on different management types (conventional or organic management) could indicate ways to decrease the concentration of pesticides in dung and thus reduce the negative effects on insects and consequently on animals, such as birds and bats, feeding on these insects.

To examine the presence of pesticides in animal feed and dung as well as the subsequent effects of the excreted pesticides on emerging insects in dung pats, we performed a monitoring study on eight dairy farms. We collected the feed and dung from these farms, and then we analysed these for different pesticides and counted the emerging insects in artificial dung pats in a field experiment. To include the effect of a pour-on application for flies, a second experiment was carried out in which we spiked the dung with different levels of deltamethrin (mimicking gradual faecal excretion of pyrethroids after pour-on applications) to study the effects on emerging insects in dung pats in the field. This study had the following objectives:

1) Measuring the pesticides present in different types of dairy cow feed and calculating pesticide intake in dairy cow rations, followed by measuring the pesticide concentration in dung and testing the effect on emerging insects in artificial dung pats in a field trial.
2) Examining the effect of the insecticide deltamethrin, which was used in different concentrations to spike dung, on emerging insects in artificial dung pats in a field trial.

We hypothesise (i) that the feed used for dairy cows contains pesticides, but that there is a difference between different types of feed and between conventional and organic farms, (ii) that pesticides in feed will be partly excreted in dung, (iii) that the presence of pesticides in dung pats will lead to a decrease in the number of insects feeding on these dung pats, and (iv) that the number of insects (Diptera and Coleoptera) will decline in dung spiked with deltamethrin, depending on the concentration.

2. Material and methods

2.1. Study site

Two experiments were carried out in June and July 2020 in a field of the experimental farm KTC Zegveld (N52° 08 26.1, E4° 50 19.6), which is situated on a drained peat soil in the western peat region of the Netherlands (37.5% soil organic matter, 18% sand, 25% clay). The field was permanent grassland with a combined grazing and cutting management under a conventional fertiliser regime with slurry manure and artificial fertiliser.

During the study, weather conditions were recorded by an automated weather station. The mean temperature was 17.0 °C (minimum 8.5 °C, maximum 30.9 °C), and average weekly precipitation was 20.4 mm (2.9 mm per day; between 0 and 17.5 mm per day). Precipitation was higher than average (long-term monthly averages are 68 mm for June and 74 mm for July (https://www.knmi.nl/nederland-nu/klimatologie/maand-en-seizoensoverzichten), and the temperature was higher than average in June (17.5 vs. 15.6 °C) and lower than average in July (17.0 vs. 17.9 °C).

2.2. Experiment 1: Effect of ration on pesticides and insects in dung

2.2.1. Farms, rations and dung collection

For this study, we selected eight dairy farms with a grass-based ration in the western peat district of the Netherlands. All rations of these farms contained fresh grass and/or grass silage and concentrates. Other components of the ration are described in Table 1. Six of the eight farms (Farms A-F) had a conventional management and two farms had an organic management (Farms G and H). Farm A had recently used a commercial permethrin pour-on against ticks and flies (Tectonik®) whereas the other farms had not used insecticides, herbicides, fungicides or anti-parasite agents in the six months prior to collection of the feed and dung samples.

On 22 June 2020, samples were taken of all the types of feed in the ration of the lactating dairy cows. In addition, 12 kg of dung was collected from the dairy cows directly after defecating to prevent contamination of dung with urine. Dung was homogenised and two subsamples were taken. One of the dung subsamples was analysed for dry matter and crude ash by Eurofins-Agro (Wageningen). The dry matter content was determined by oven drying at 103 °C for four hours (ISO 6496) and ash content by incineration at 550° for four hours. The other dung subsample and feed samples were analysed for pesticides by Eurofins Zeeuws Vlaanderen. For the pesticide/residue analysis, all feed and dung samples were freeze-dried and homogenised prior to analysis. Homogenised samples were extracted with acetone, petroleum ether and dichloro-methane, using an optimised mini-Luke method. A total of 664 pesticides and pesticide residues were analysed with gas chromatography (Agilent), liquid chromatography (LC-chromatograph

Table 1
Rations of the lactation group on the selected dairy farms.

	Farm	# Feeds sampled	Types of feed in the ration	Organic	Pour-on
1	A	7	Grass, grass silage (2x), potatoes, concentrates (2x)	N	Y
2	B	7	Grass silage, haylage, concentrates (2x), brewers grains, grass pellets, carbon powder	N	N
3	C	6	Grass, barley, beet pulp, hay 2019, corn pellets, hay 2020	N	N
4	D	4	Grass, grass silage, beet pulp, concentrates	N	N
5	E	2	Grass, concentrates	N	N
6	F	3	Grass, concentrates, potatoes	N	N
7	G	2	Concentrates, grass silage	y	N
8	H	3	Grass (2x), concentrates	Y	N

(Agilent) and MSMS (Sciex)). The detection limit (LOD) was 0.1 mg per kg sample (Van Eekeren et al., 2022). Based on the ration of the animals, intake of different pesticides was estimated via the following formula:

Daily pesticide intake per animal per day = DMI_{f1} X $conc_{f1p1}$ + DMI_{f2} X $conc_{f2p1}$ + ….

In which DMI_{fx} = dry matter intake of Feed x and $concentration_{fxpy}$ = concentration of pesticide y in feed x.

2.2.2. Design of experiment 1 with artificial dung pats

The remaining cattle dung was used to make artificial dung pats on the grassland field at KTC Zegveld in order to investigate the effect of different types of dung on the presence of invertebrates. Experiment 1 was conducted for two weeks in June and July 2020. A 50 m x 20 m area of the grassland was fenced to keep the cattle out. In this area, 4 plots of 2 m x 10 m were demarcated, situated at least 5 m from each other. The day before the pats were placed, grass was mowed at 5 cm grass length. In each of the four plots, an artificial dung pat from each of the eight dairy farms was randomly allocated, adding up to a total of 32 artificial dung pats. Artificial dung pats were formed by pouring the dung (2 kg) into a circular collar with a diameter of 26 cm. They were placed on squares of plastic netting (mesh size 2 cm) to facilitate later retrieval, and the pats were covered by wire netting (mesh size 4 cm) to prevent birds or mammals from destroying them.

The pats were retrieved after 7 days or 14 days (plots 1 and 3 on day 7, plots 2 and 4 on day 14; 16 pats per sampling occasion), weighed and placed in labelled plastic bags. Subsequently, they were transported to the laboratory, where they were processed on the same day. The dung pats were divided into four quarters, and two opposite quarters were stored at −20 °C until DM analysis. The two other quarters were soaked in water, after which all invertebrates (insects and larvae) were removed from the pats by hand. The invertebrates were stored in 70% ethanol at 5 °C until determination of the species or genus (Skidmore, 1991). Then the number of invertebrates per subsample was recalculated to the average subsample weight (25% of dung pat; 500 g fresh material on day 0).

2.3. Experiment 2: Effect of deltamethrin on insect abundance

2.3.1. Cows, ration and dung collection

On two consecutive mornings (3 and 4 June 2020), 150 kg of fresh dung was collected from the lactating group on the experimental farm KTC Zegveld. The dung was collected directly after defecating, to prevent contamination of dung with urine. The cows in the lactation group were fed a basic ration of fresh grass, grass silage, potatoes and concentrates. The animals had never been treated with insecticides.

2.3.2. Treatments

The collected dung was homogenised by thorough mixing before being used. Subsequently, two samples were taken, one was analysed for dry matter and crude ash by Eurofins-Agro (Wageningen) and the other was analysed for pesticides by Eurofins Zeeuws Vlaanderen. The methodology used was the same as for Experiment 1.

The rest of the dung was equally divided over four 100-L bins to prepare it for the four treatments. A commercial deltamethrin product (Butox® Swiss, MSD Animal Health, 7.5 mg ml^{-1} pour-on suspension) was used to create dung artificially contaminated with insecticide, i.e. by 'spiking' it. The deltamethrin product was mixed into the dung to produce 35 kg batches of dung with aimed concentrations of 0 (control), 50, 200 and 1000 μg deltamethrin per kg dung on dry matter (DM) basis. These concentrations were chosen based on published studies with deltamethrin (Wardhaugh et al., 1998, 2006; Sands et al., 2018), and they were expected to mimic the excretion pattern of deltamethrin in dung in the two weeks after pour-on applications. For each concentration, the appropriate amount of deltamethrin was transferred to a beaker using a pipette and then water was added to 100 ml. Water and deltamethrin were mixed thoroughly before being poured into the dung while mixing. The dung was homogenised by mixing for a further two minutes to make sure that the insecticide was evenly distributed throughout. For the control batch, only 100 ml of water was added to the dung, and subsequently the control dung was treated in exactly the same manner as the other treatments.

After correction for the dry matter content (11.3%) of the dung, the actual concentrations of deltamethrin were 0 (treatment I, control), 60 (treatment II), 270 (treatment III) and 1333 (treatment IV) μg kg^{-1} DM. Analysis of the dung on pesticides showed that other pesticides were also present in the dung (see Supplementary Materials I).

2.3.3. Design of field experiment with artificial dung pats

Experiment 2 was conducted for five weeks in June and July 2020. A 50 m x 20 m area of the grassland was fenced to keep the cattle out, which is the same as in Experiment 1. In this area, four plots of 2 m x 10 m were demarcated, and situated at least 10 m from each other. Before establishment of the dung pats, the grass was mowed at 5 cm height. In each of the four plots, 16 artificial dung pats, four of each treatment, were positioned, adding up to a total of 64 artificial dung pats. The four treatments were randomly distributed per plot. Pats were formed in the same way as in Experiment 1.

2.3.4. Dung pat sampling and analysis

From each plot, one experimental pat per treatment was harvested for analysis on day 7, day 14, day 21 and day 35. Hence, 4 pats of each type of dung were removed on each sampling occasion, leading to a total of 16 pats per sampling occasion. The order of pats to be removed per plot was randomly assigned at the start of the trial. The wet weight of each pat was recorded in the field, and the pats were transported to the laboratory in labelled plastic bags. Samples were processed in the same way as in Experiment 1.

2.3.5. Invertebrates on site

To describe the insects and other invertebrates regularly found at the test location, insect traps were placed on 18 June 2020 on both sides of four of the demarcated plots, resulting in a total of 8 traps. Traps consisted of a cup with a diameter of 7 cm and a height of 10 cm. The cups were buried, such that the rim of the cup was at the surface of the soil. Cups were filled with a solution of salted water to preserve the insects, and detergent was added to reduce surface tension. A plastic plate was placed over the trap to prevent rain from entering. One week later, on 25 June, the insects captured were collected and stored in the salt-water solution at 5 °C until identification to genus or species level.

The average number of Diptera in the traps was 13.9 per trap. Diptera were mostly unidentified, due to preservation effects on the small and fragile Diptera. The average number of Coleopterae was 34.5 per trap, and the main family present was the Carabidae (ground beetle) (on average 26.4 per trap). The second largest group of beetles was the Staphylinidae (rove beetle), with on average 4.5 animals per trap present. Of the other orders, spiders (on average 142 per trap) and Collembola (on average 30.9 per trap) were the main invertebrates present in the insect traps. More details can be found in Supplementary materials II.

2.3.6. Earthworms

On day 14 (25 June), earthworms and other invertebrates (including larvae) were sampled below the dung pats by digging out a soil block of 20 × 20 × 10 cm. Earthworms were hand-sorted, counted, weighed and fixed in 70% ethanol prior to identification. Numbers and biomass were expressed per m^2. Worms were classified as adults or juveniles, identified to species and classified into functional groups (epigeic, endogeic and anecic species) (Bouché, 1977). Invertebrates were stored immediately in 70% ethanol at 5 °C until species or genus determination.

2.4. Statistical analysis

Experiment 1 was set up as a monitoring study to observe the effects of different types of dung. Abundance data of dung organisms in conventional and organic management were analysed with ANOVA. Log transformation (10 log (1 +x)) was used to obtain normality, and Spearman correlations were calculated with the statistical package R. For Experiment 2, abundance data of dung organisms were analysed with two-way ANOVA, using deltamethrin treatment and sampling date of dung pats after exposure in the field as fixed factors. Here, data were also log-transformed (10 log (1 +x)) to obtain normality. ANOVAs were performed in Genstat for Windows (VSN International, 2019). In both experiments, due to the low numbers of adult Diptera and adult Coleoptera, dung invertebrates were analysed statistically per order.

3. Results

3.1. Experiment 1

3.1.1. Pesticides in different types of feed

A total of 70 pesticides were detected in the different feed samples on the eight farms (see Table 2 for the numbers of pesticides per feed and Table 3 for the most common pesticides). Some pesticides were detected more frequently or in higher concentrations than others. Grass and other forage products (fresh grass, grass hay, grass silage) had lower concentrations of the different pesticides than wet by-products (potatoes, pressed beet pulp and brewers grains), concentrates or barley. The average number of pesticides in the 15 grass product samples was 9.8, with a minimum of 8 and a maximum of 16. The highest concentration of a specific pesticide (phthalimide) was 48 μg kg^{-1} DM in sampled fresh grass. The herbicide chlorpropham was analysed in all 15 grass products samples; however, concentrations were low, with an average of 12.2 μg kg^{-1} DM. Other pesticides recovered in several samples of grass products were DEET, anthraquinone, diphenyl, phenylphenol-2, phthalimide and prosulfocarb.

In all wet by-products (potatoes, pressed beet pulp, brewers grains), chlorpropham was detected, with highest concentrations in potatoes (average of 358 μg kg^{-1} DM). In brewers grains, cypermethrin and piperonyl butoxide were higher (1393 and 681 μg kg^{-1} DM, respectively) than in the wet by-products or in concentrates. On average, there were 13 pesticides per wet by-product sample, varying from 7 (potatoes) to 25 (brewers grains).

A total of 14 different concentrates and raw materials (barley, grass pellet and carbon powder) were sampled. In concentrates, approximately 16 different pesticides were detected per sample. The most commonly observed pesticides in concentrates and raw materials were anthraquinone, chlorpropham, cypermethrin, difenoconazole, phthalimide, piperonyl butoxide, pirimiphos-methyl and tebucanazole. The concentration of pesticides in concentrates was lower in the organic than in the conventional samples.

Table 3
Most common pesticides in sampled animal feed (34 samples). Only concentrations > 10 μg kg^{-1} DM or frequently found pesticides are reported. A list of all pesticides detected in sampled animal feed (average over rations) is given in Supplementary materials III. Av = Avicide, F = Fungicide, H = Herbicide, I = insecticide, Syn = synergist.

		# samples	Average concentration (μg kg^{-1} DM)	Values above average in: (with concentration in μg kg^{-1} DM given in brackets)
Anthraquinone	Av	22	6.6	Carbon powder (56), Grass pellet (37)
Chlorpropham	H	33	35	Potatoes (375), Pressed pulp (163)
Cypermethrin	I	13	135	Brewers grains (1393), Concentrates (up to 182)
Cyproconazole	F	10	1.8	Pressed pulp (5.7)
DEET	I	15	1.7	Grass (ext) (3.7)
Dichloran	F	1	131	Concentrates (131)
Difenoconazole	F	20	3.2	Pressed pulp (18)
Diphenyl	F	11	8.1	Grass silage (13)
Epoxiconazole	F	10	14	Concentrates (46)
Fenpropidin	F	3	20	Pressed pulp (34)
Mecoprop	H	1	61	Grass pellet (61)
Permethrin (C+T)	I	1	39	Grass (39)
Phenylphenol-2	F	16	2.6	Grass pellet (15)
Phthalimide (folpet)	F	25	51	Grass pellet (590), Concentrates (up to 106), Brewers grains (71), Carbon powder (52)
Piperonyl butoxide	Syn	20	147	Concentrates (882), Brewers grains (681), Barley (228)
Pirimiphos-methyl	I	17	28	Concentrates (239), Brewers grains (94)
Prosulfocarb	H	13	1.9	Grass silage (3.4)
Prothioconazole-desthio	F	13	1.9	Pressed pulp (2.5)
Tebuconazole	F	14	3.1	Concentrates (21)

3.1.2. Pesticides in the ration per farm

The number of pesticides present in the ration differed per farm (Table 4). With 50 pesticides in the ration, Farm B had the highest number of pesticides. Farm G, one of the organic farms in the study, had 9 pesticides and thus the lowest number in the ration. On average, fungicides were present in the ration in the highest frequencies (11), followed by insecticides (10) and herbicides (6). There were no significant differences in the number of pesticides per type of farm

Table 2
Average concentration of chemical substances in μg kg^{-1} DM present in different types of feed. The number in brackets is the average number of pesticides, insecticides, herbicides or fungicides found per sample.

	Type of feed	# samples	Total pesticides μg kg^{-1} DM	Insecticides μg kg^{-1} DM	Herbicides μg kg^{-1} DM	Fungicides μg kg^{-1} DM	Others[a] μg kg^{-1} DM
Grass products	Grass	7	67 (10)	7.7 (2)	18 (3)	29 (3)	12 (2)
	Grass silage	5	52 (9)	4.5 (2)	21 (3)	16 (2)	10 (1)
	Grass hay	3	49 (12)	3.8 (2)	17 (4)	21 (3)	6.8 (2)
Wet by-products	Brewers grains	1	2375 (26)	1564 (8)	17 (4)	104 (11)	682 (2)
	Potatoes	2	376 (8)	3.5 (2)	358 (1)	9.8 (4)	4.5 (3)
	Pressed pulp	2	167 (13)	1 (1)	86 (2)	80 (10)	1.8 (1)
Concentrates and grain	Barley	1	331 (12)	84 (3)	0 (0)	18 (7)	229 (2)
	Grass pellet	1	725 (18)	6.1 (3)	69 (5)	611 (7)	39 (3)
	Concentrates (conventional)	9	429 (19)	123 (7)	13 (3)	69 (7)	225 (2)
	Concentrates (organic)	2	71 (7)	1.4 (2)	4.7 (2)	59 (2)	5.8 (2)
Additives	Carbon powder	1	111 (5)	0.9 (1)	1.6 (1)	52 (1)	56 (2)

[a] Others e.g. Anthraquinone, (avicide) or piperonyl butoxide (synergist)

Table 4
Number of different pesticides in the total ration per farm.

	A	B	C	D	E	F	G[a]	H[a]	Average
Herbicide	6	10	6	6	4	5	3	6	6
Insecticide / acaricide[b]	20	17	9	13	7	9	2	6	10
Fungicide	12	21	17	14	5	12	3	7	11
Avicide	1	1	1	1	1	1	1	1	1
Other[c]	2	1	0	0	1	0	0	0	1
Total	41	50	33	34	18	27	9	20	29

[a] organic farm;
[b] including synergist piperonyl butoxide;
[c] other components are caffeine and 1,4 dimethylnaphtalene (plant growth regulator/sprout suppressant)

(conventional / organic), although a trend was observed for higher numbers of insecticides (p-value = 0.07), fungicides (p-value = 0.09) and total number of pesticides (p-value = 0.07) on conventional farms compared to organic farms.

The average concentration of pesticides ingested per cow per day was calculated based on the ration (Table 5). The highest calculated intake per cow was on Farm E, which had a low number of pesticides in the ration but a very high total intake due to high concentrations of cypermethrin, piperonyl butoxide and pirimiphos-methyl. Farm C (conventional) and Farm H (organic) had the lowest average calculated intake. Anthraquinone (avicide), chlorpropham (herbicide/sprout suppressant) and difenoconazole (fungicide) were present in the ration of all farms. Cypermethrin, DEET, phthalimide, piperonyl butoxide, pirimiphos-methyl and tebucanazole were found on seven farms. The highest calculated amounts of pesticides in the rations were observed for chlorpropham, cypermethrin, phthalimide, piperonyl butoxide and pirimiphos-methyl. There were no significant differences in the sums of concentration of pesticides in the ration per type of farm (conventional/ organic); this was mainly due to the high variation in the sums of concentrations on the conventional farms.

3.1.3. Pesticides in dung

The number of pesticides recovered in dung varied between 3 and 8 per farm (Table 6). The highest concentration of pesticides (mainly permethrin) was found in the dung from Farm A. On this farm, three days before sampling, the cows had been treated with a permethrin pour-on against flies. The lowest concentrations of pesticides were found in dung from Farms C (conventional) and H (organic); the latter also had the lowest intake of pesticides. A total of 13 different pesticides were recovered in the dung from the eight farms (Table 6). No significant differences were observed in concentrations of pesticides in dung per type of farm (conventional/organic), which was mainly due to the high variation on the conventional farms.

Table 5
Average intake of the different pesticides per cow per day in μg kg^{-1} DM. Pesticides are only included in this table if they had concentrations > 50 μg per day or had been detected on four farms or more. A complete list of all pesticides detected (average over ration) is given in Supplementary materials III. A = Acaricide, Av = Avicide, F = Fungicide, H = Herbicide, I = insecticide, Syn = synergist.

Compound	Type	A	B	C	D	E	F	G[b]	H[b]	#	Mean
2_4-D	H		73	0	10		4		2	5	18
Anthraquinone	Av	16	62	27	38	10	34	66	50	8	38
Caffein	Rest	386	10			172				3	189
Chlorpropham	H	436	142	318	92	235	836	356	163	8	322
Cypermethrin	I	141	847	6	350	908	17		14	7	326
DEET	I	20	11	13	11		12	17	53	7	20
Deltamethrin	I	99	192	52	16	198	140			6	116
Dichloran	F						788			1	788
Difenoconazole	F	15	7	19	37	22	36	17	30	8	23
Diphenyl	F	150		16		144		232		4	136
Diphenylamine	F		14		12				95	3	40
Epoxiconazole	F	74	9	23	50	202	35			6	65
Fenpropidin	F	5		23	51					3	27
Flonicamid-TFNA	I	1		8	31		68			4	27
Haloxyfop	H	12	18		18	19				4	17
Imidacloprid	I	6		11	8	11				4	9
MCPA	H		3	15			11		37	4	17
Mecoprop	H		55							1	55
Permetrhin-cis	I	144								1	144
Permethrin-trans	I	258								1	258
Phenylpenol-2	F	2	45	32	14		20		52	6	28
Phthalimide	F (M)	162	1082	368	555		320	825	496	7	544
Piperonyl butoxide	Syn	857	928	191	288	4412	1018		53	7	1107
Pirimiphos-methyl	I, A	119	134	11	42	1196	18		27	7	221
Prosulfocarb	H	11	38	11	12			27	31	6	22
Prothioconazole-desthio	F		15	19	21		24		30	5	22
Pyridalyl	I							128		1	128
Tebuconazole	F	33	30	4	8	18	9		6	7	15
Tefluthrin	I	4	1	5		20	6			5	7
Terbuthylazine	H	25	4			46	23		29	5	26
Terbuthylazine-desethyl	H	73	7				56		74	4	53
Tetraconalole	F		2	2	5	13	6			5	6
Other pesticides[a]		177	142	117	213	5	57	0	14		
Herbicide		573	390	391	159	301	930	382	336		
Insecticide / Acaricide		1754	2145	298	861	6749	2082	145	156		
Fungicide		493	1265	574	823	399	492	1074	713		
Avicide		16	62	27	38	10	34	66	50		
Other[c]		389	10	0	0	172	0	0	0		
Total		3226	3871	1291	1882	7631	3538	1668	1256		

[a] pesticides with concentrations < 50 μg day^{-1};
[b] organic farm;
[c] other components are caffeine and 1,4 dimethylnaphtalene (plant growth regulator/sprout suppressant)

Table 6
Pesticides present in dung (μg kg^{-1} DM) on the eight farms, and significant correlation with ingested compounds (r = correlation coefficient, and p = p-value).

		Farm										Correlation with ingested compounds	
Compound		A	B	C	D	E	F	G	H	#	Mean	r[~]	p
Anthraquinone	Av.		16	9	11	8	14	13	11	7	12	0.705	0.022
Chlorpropham	H	25	44	34	40	25	73	26	29	8	37		
Cypermethrin	I	23	59		97	110	21			5	62	0.952	< 0.001
Diphenyl	F	67	79	84	62	67	80	82	72	8	74		
Fenpropimorph	F	12								1	12		
Fluxapyroxad	F/B		18					13		2	15		
Isopyrazam	F							15		1	15		
Metalaxyl	F		6							1	6		
Permethrin-cis*	I	455								1	455		
Permethrin-trans*	I	1398								1	1398		
Phenylphenol-2	F				16					1	16		
Piperonyl butoxide	Syn		62				50			2	56		
Tebuconazole	F		42					32		2	37		
Total		1980	310	127	226	210	238	181	112		423	0.762	0.007

* Animals were treated with permethrin 3 days before dung collection;
~ Spearman's rank correlation

The most frequently analysed pesticides in dung were chlorpropham (all samples), diphenyl (all samples) and anthraquinone (7 of the 8 samples). Cypermethrin was found in most of the feed samples; this insecticide was found in the highest concentrations in five dung samples. Pirimiphos-methyl was also found in the feed samples on five farms, but was not found in dung at all. Piperonyl butoxide was found in the feed samples of seven farms, but only found in the dung from two farms. Diphenyl was found in all dung samples although it was only ingested in four of the rations. Furthermore, isopyrazam was found in the dung, but not in the different types of feed or in the ration. For the pesticides present in the dung of more than two farms, the relationship was calculated with the ingested pesticides. Significant correlations were calculated for anthraquinone, cypermethrin and total pesticides (Table 6).

3.1.4. *Invertebrates in dung and relationship with pesticides*

Per 500 g freshly deposited dung, the average number of Coleoptera larvae varied from 0 to 16 and the average number of Coleoptera adults varied from 2 to 22. The adults were classified to belong mainly to the families of Staphylinidae (70%) and Scarabaeidae (including the genus *Aphodius*) (7%). Significantly higher numbers of Coleoptera larvae and Coleoptera adults were found in the dung from the organic farms compared to conventional farms (p-value type < 0.05; Table 7). Low numbers of Coleoptera larvae and adults were observed in the dung from Farms A, B, D and F (Fig. 1a).

Numbers of Diptera larvae in dung were higher than those of Coleoptera (Table 7 and Fig. 1). Besides Diptera larvae and adults, Diptera pupae were also counted. No significant difference was found in the number of Diptera between farm types. The dung of Farm A, the farm with permethrin pour-on treatment, showed a significant trend for a higher number of pupae compared to the other farms (on average 71 with pour-on for Farm A vs. 29 without pour-on for the other farms; p-value = 0.054). A significant negative correlation was observed between the concentration of cypermethrin and the number of Coleoptera (larvae and adults) (p-value < 0.05; Table 8). No significant correlations were observed between concentrations of pesticides and the number of Diptera, apart from the negative correlation of piperonyl butoxide with Diptera adults ($r = -0.54$; p-value = 0.045). The pesticides anthraquinone, chlorpropham and tebuconazole did not show significant correlations with any of the invertebrates and have therefore been excluded from Table 8. Permethrin-cis and permetrin-trans did not show significant correlations either, as these products were only present in one sample. Conventional farms (Type) were significantly positively related with pesticides in dung ($r = 0.63$; p-value = 0.009), and negatively with Coleoptera (adults) ($r = -0.69$; p-value = 0.005).

Table 7
Number of Coleoptera (larvae and adults) and Diptera (larvae, pupae and adults) per 500 g freshly deposited dung of conventional and organic farms.

Invertebrates	Type		P-value		
	Conventional	Organic	Type	Day	Type x Day
Coleoptera larvae	2.30[a]	6.76[a]	0.012	0.005	0.033
Coleoptera adults	9.1[b]	16.9[a]	0.002	0.027	NS
Sum Coleoptera	11.4[b]	23.7[a]	0.002	NS	NS
Diptera larvae	151	143	NS	< 0.001	NS
Diptera pupae	20.9	16.5	NS	< 0.001	NS
Diptera adults	2.41	2.58	NS	0.005	NS
Sum diptera	174	162	NS	< 0.001	NS

Means in the same row and on the same day with different superscripts differ significantly (a, b: p-value < 0.05, based on log(10)(x + 1) transformation)

3.2. *Experiment 2*

3.2.1. *The presence of invertebrates in dung and how this relates to deltamethrin*

The variation in the number of invertebrates in dung spiked with deltamethrin was high (Figs. 2 and 3). The number of Coleoptera larvae was highest in treatment I (control) and lowest in treatment IV, but this was not statistically significant (p-value treatment = 0.227; Table 9). The number of Coleoptera adults (mainly Staphylinidae) was significantly highest in treatment I and lowest in treatment IV (p-value treatment = 0.049). The sum of Coleoptera was significantly highest in treatments I and II, and lowest in treatment IV (p-value treatment = 0.021). At the same time, higher numbers of Diptera (pupae, larvae and adults) were observed in the higher deltamethrin treatments (III, IV) compared to the control (I) and lowest concentration (II), but these differences were not significant (Table 9). Differences were only significant for Diptera pupae on day 14 (p-value treatment = 0.017) and day 21 (p-value treatment = 0.032) (Fig. 3). A trend was observed for Diptera larvae on day 14 (p-value treatment = 0.089).

The concentration of deltamethrin was negatively correlated with the number of Coleoptera adults ($r = -0.27$; p-value = 0.031; Supplementary materials IV), but it was positively correlated with total Diptera ($r = 0.28$; p-value < 0.05). More correlations were observed when analysing separately per day. On day 7, the correlation between deltamethrin and Coleoptera adults was negative ($r = -0.56$; p-value = 0.024) and on day 14, the correlation between deltamethrin and Diptera larvae was positive ($r = 0.64$; p-value = 0.008). Subsequently, on day 21, the concentration of deltamethrin was positively correlated to Diptera pupae ($r = 0.68$; p-value = 0.004) and on day 35 negative correlations were observed between deltamethrin and Coleoptera larvae ($r = -0.67$; p-value = 0.005), Coleoptera adults ($r = -0.69$; p-value = 0.004) and Diptera larvae ($r = -0.56$; p-value = 0.025).

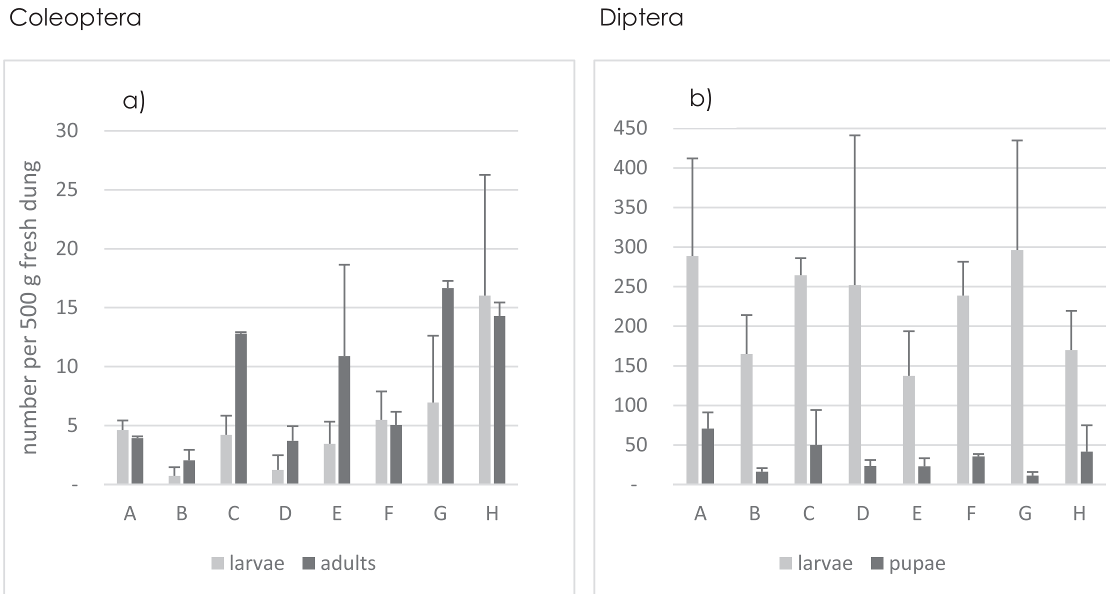

Fig. 1. a) Number of Coleoptera (larvae and adults) and b) Diptera (larvae and pupae) per 500 g fresh dung in dung pats on day 14 of the experiment (error bars = se; n = 2).

Table 8
Significant Spearman correlations between different invertebrates and pesticide concentrations in dung on day 14 in Experiment 1. Significance (p-value) in brackets.

	Coleoptera			Diptera			
	Larvae	Adult	Sum	Larvae	Pupae	Adult	Sum
Pesticides							
Cypermethrin	-0.52 (0.037)	-0.53 (0.034)	-0.65 (0.007)				
Isopyrazam							
Metalaxyl		-0.53 (0.034)	-0.53 (0.034)				
Piperonyl butoxide		-0.51 (0.045)				-0.54 (0.032)	
Sum insecticides		-0.52 (0.039)	-0.57 (0.022)				
Sum total		-0.67 (0.005)	-0.69 (0.003)				

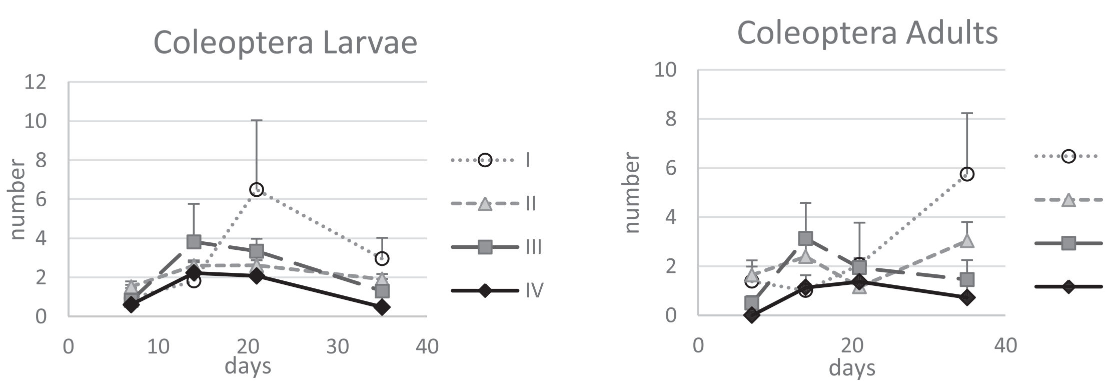

Fig. 2. Coleoptera (larvae and adults) in dung pats (per 500 g fresh dung) of Experiment 2 (error bars = se, n = 4). 0 (I), 60 (II), 270 (III) and 1333 (IV) μg deltamethrin kg^{-1} dry matter.

Earthworms were counted in the grass sod under the dung on day 14 of Experiment 2. On average, we found 227 ± 56 earthworms per m^2 in the soil layer of 0–10 cm for the four treatments. These were mostly *Lumbricus rubellus* (adults 155 ± 96; juveniles 35 ± 31), but we also found *L. castaneus* (adults 2 ± 4; juveniles 2 ± 4), *Allolobophora chlorotica* (adults 2 ± 4; juveniles 5 ± 10) and *Aporrectodea caliginosa* (28 ± 23). No significant differences were observed between the treatments. Furthermore, some Coleoptera (larvae and adults) and Diptera larvae were also found in the grass sods. Per m^2, we found for the four treatments on average 58 ± 40 Coleoptera larvae and 55 ± 42 Diptera larvae, as well as 6.3 ± 4.5 *Aphodiinae* adults and 6.3 ± 5.0 *Staphylinidae* adults. No significant differences were observed between the four treatments.

4. Discussion

In our study we aimed to cover the full route from pesticide contamination in the feed via the cow and the dung to insect survival in dung pats. We found that pesticides are widely present in the feed for dairy cows in the Netherlands. We even found pesticides in feed on

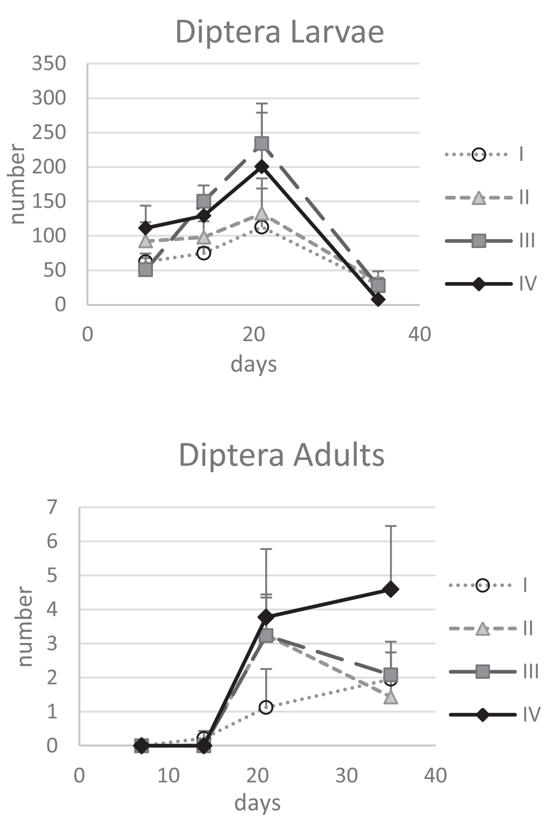

Fig. 3. Diptera (larvae, adults and pupae) in dung pats (per 500 g fresh dung) in Experiment 2 (error bars = se, n = 4). 0 (I), 60 (II), 270 (III) and 1333 (IV) μg deltamethrin kg^{-1} dry matter.

Table 9
Number of Coleoptera (larvae and adults) and Diptera (larvae, pupae and adults) per 500 g freshly deposited dung, treated with 0 (I), 60 (II), 270 (III) and 1333 (IV) μg deltamethrin kg^{-1} dry matter (analysed with ANOVA), 7, 14, 21 and 35 days after deposition.

Invertebrates	Treatments				P-value		
	I	II	III	IV	Treatment	Day	Treatment *Day
Coleoptera larvae	3.0	2.2	2.3	1.3	NS	0.002	NS
Coleoptera adults	2.6^{a}	2.1^{ab}	1.8^{ab}	0.8^{b}	0.049	0.091	NS
Sum Coleoptera	5.6^{a}	4.2^{a}	4.1^{ab}	2.2^{b}	0.021	0.003	NS
Diptera larvae	70	89	115	112	NS	< 0.001	NS
Diptera pupae	25.3	26.2	37.7	51.5	NS	< 0.001	0.034
Diptera adults	0.8	1.2	1.3	2.1	NS	< 0.001	NS
Sum Diptera	96	116	154	166	NS	0.027	NS

Means in the same row and on the same day with different superscripts differ significantly (a, b: p-value < 0.10, based on log(10)(x + 1) transformation).

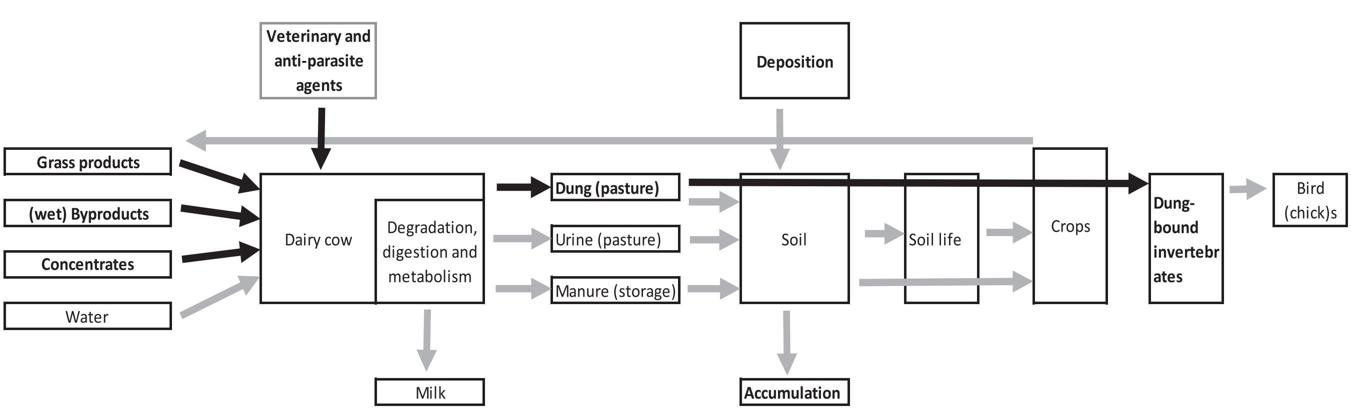

Fig. 4. A schematic overview of the route of pesticides on a dairy farm. Bold text and black arrows were measured in this study; regular text and grey arrows are assumed.

organic farms, although mostly with lower concentrations. Pesticides were found in all types of feed, and this underlines the fact that feed intake can be an important source of pesticides in the environment. While some of the pesticides can be degraded during digestion, other more persistent pesticides are transferred to the dung of the dairy cows and can have a toxic effect on emerging dung-bound insects. Fig. 4 presents a schematic overview of the potential distribution route of pesticides on a dairy farm. The different steps in this route are discussed in the next few sections.

4.1. Pesticides in feed

We hypothesised that feed used for dairy cows contains pesticides, and indeed we detected pesticides in all types of feed in our study. Concentrations were mostly higher in concentrates and by-products than in grass products. The two organic concentrates had lower concentrations of pesticides than the conventional concentrates. This confirms research by Buijs and Samwel-Mantingh (2019) and Buijs et al. (2022), who also found lower concentrations of pesticides on organic farms. The detected pesticides in concentrates may have several sources. They could have been used during cultivation for crop protection, used to prepare a field for a new crop or used during storage or transport (Ortelli et al., 2005). In our study, especially by-products (brewers grains, pressed pulp and potatoes) were found to have potentially high concentrations of pesticides; our sample of brewers grains contained 1393 $\mu g\ kg^{-1}$ DM cypermethrin. This is higher than the results of Walorczyk and Drożdżyński (2012), who found up to 110 µg cypermetrhin per kg product, mainly in wheat and malt, the precursors of brewers grains.

Although the concentration of pesticides in grass and grass products was lower than in concentrates and by-products, none of the grass products were free of pesticides and their residues; this was not even the case on the organic farms, and this finding is in contrast with findings by Mol et al. (2014), who collected hay samples in the Netherlands, and no pesticides were reported in 65% of the 43 samples. However, the limits of detection were higher (mostly $<10\ \mu g\ kg^{-1}$, always $< 50\ \mu g\ kg^{-1}$) than in our study (0.4–2.4 $\mu g\ kg^{-1}$, depending on type of feed and the dry matter content of the sample). In our study, pesticide concentration in grass products was also relatively low ($<67\ \mu g\ kg^{-1}$ DM). The low pesticide concentration in grass products was in sharp contrast to the more than ten-fold higher concentrations in grass pellets (725 $\mu g\ kg^{-1}$ DM in total, phthalimide (Farm F; 590 $\mu g\ kg^{-1}$ DM) and mecoprop (Farm H; 61 $\mu g\ kg^{-1}$ DM) being the pesticides with the highest concentrations). Grass pellets are artificially dried and may originate from another location, possibly even from grasslands in rotation with arable land on which more pesticides are used, which could explain these contrasting values.

The insecticide permethrin was only detected in fresh grass, sampled on Farm A. This is also the only farm that used permethrin as a pour-on on the cows; as a result, there were high concentrations in dung. Therefore, the concentration of permethrin in the grass of Farm A probably originated from previous treatments of the cattle and subsequent spreading of the manure on the land, according to the pesticide route in Fig. 4. Pyrethroids and their degradation products are known to be relatively stable and to degrade slowly, especially in soils with a high clay content or a large percentage of organic matter (Kaufman et al., 1981), which is the case on the peat soils in our study.

We also detected chemical substances in our samples that are currently banned in agriculture in the EU, such as anthraquinone, chlorpropham, DEET, diphenyl and phenylphenol-2. The presence of these pesticides might be explained by the import of products from outside the EU, historical use, deposition or surface water contamination. Anthraquinone and DEET were both present in low concentrations, but with high frequencies; these two substances have been prohibited for agricultural purposes for decades. Anthraquinone may have been present as a consequence of deposition due to poor combustion of fossil fuels. Previous research in soils demonstrated a relation with distance to highways (Van Eekeren et al., 2022). For DEET, concentrations in grass may be a result of historical use, but sewage or surface water contamination due to anthropogenic use are more likely sources of DEET pollution (Emissieschattingen diffuse bronnen, 2018).

Diphenyl is also prohibited as a plant protection product (not as biocide), but it can be produced in industry (Boehncke et al., 1999) and by wood burning (Avagyan et al., 2016). Chlorpropham and phenylphenol-2 were allowed for agricultural purposes in the Netherlands until June 2020, and concentrations in feed may therefore originate from agricultural use. Chlorpropham was found in most rations, in all dung and grass products samples, and will thus possibly enter the farm via wet by-products and concentrates, and spread on grasslands via dung and manure, causing pollution of the grass. Chlorpropham was especially high in potatoes (340 – 375 $\mu g\ kg^{-1}$ DM), where it was widely used as a sprout suppressor. Chlorpropham has been prohibited in the EU since 2020.

4.2. Pesticide excretion in dung

We hypothesised that pesticides in the ration of dairy cows are at least partly excreted in dung. As shown in Tables 5 and 6, the number of pesticides ingested ranged from 9 to 50 per ration and total (cumulative) concentrations ranged from 1260 to 7630 $\mu g\ day^{-1}$. Incidence of pesticides in dung was lower, with 3–8 pesticides per dung sample (Table 7). These numbers of pesticides are lower than the numbers reported by Buijs and Samwel-Mantingh (2019), who used manure from storages instead of dung freshly from the cow; they detected 15 different pesticides per sample on average. However, in Experiment 2 (Supplementary materials I), more pesticides (#17) were detected in dung than in Experiment 1, even though the total summed concentration (178 $\mu g\ kg^{-1}$ DM) was not higher than in Experiment 1 (112–310 $\mu g\ kg^{-1}$ DM; excluding Farm A). The higher number of detected pesticides in Experiment 2 was probably due to the higher amount of dung sampled in Experiment 2 compared to Experiment 1 (150 kg and 12 kg, respectively), in combination with the low detection limit.

For cypermethrin, chlorpropham and anthraquinone, a clear positive relationship was found: the higher the intake in the ration, the higher the excretion in dung. This indicates that these products are only partly degraded by the animals and that feed containing these pesticides will pose a risk to the environment. The fact that not all pesticides were recovered in the dung indicates that some pesticides are metabolised in the intestinal tract, are excreted in urine and/or milk, or stay as a residue in the animal body (Akhtar et al., 1992). The degradation path of the different types of pesticide may depend on micro-organisms, temperature, pH, humidity, and other aspects (Wohde et al., 2016). An example of a pesticide which is possibly degraded is piperonyl butoxide; it was only detected in low concentrations in two dung samples while it was in the ration on seven farms. It has been observed that in rats most of the administered dose is excreted in faeces (Byard and Needham, 2006), but the monogastric digestive system of rats is different from the gastro-intestinal tract of dairy cows. An example of a pesticide which is possibly excreted in urine is pirimiphos-methyl; it was not recovered in dung and was calculated to be ingested on seven of the eight farms. According to EFSA (EFSA Scientific report, 2005), pirimiphos-methyl is extensively metabolised in the body, and most metabolites are excreted in urine.

Additionally, some pesticides were detected in the dung but had not been measured in the different types of feed or were present in dung in higher concentrations than might be expected from the feed; this indicates that there were pesticides in the dung originating from other sources than the feed intake. Diphenyl was detected in the dung samples of all eight farms while it was calculated to be present in only four of the rations. This indicates that feed intake is not the only source of diphenyl in the dung samples. The average dung excretion per cow per day is about 5 kg DM, so the average diphenyl excretion can be calculated to be approximately 350 μg per day, which is much higher than the average intake (136 μg per cow per day, on the 4 farms where it was recorded). This discrepancy might be caused by sampling errors (another batch of feed, inhomogeneous samples), but this seems unlikely as it was observed on all farms. Drinking water was not tested, and this may also have contained chemical substances, especially if surface water had been used, which is common practice during the grazing season in the peat areas in the western part of the Netherlands. Therefore, in future research the drinking water (surface water) should also be taken into account. Permethrin was excreted in the dung on Farm A in higher amounts than it was ingested, due to the pour-on that was applied three days before the collection date. The concentration in dung will gradually decrease in the time after application, as is also shown for deltamethrin (Venant et al., 1990) and cypermethrin (Virlouvet et al., 2006).

4.3. Effect of pesticides in dung samples on invertebrate presence

To investigate whether the pesticides present in the dung samples from eight farms had an effect on the presence and survival of insects, a field trial with artificial dung pats was conducted. We found a significant negative correlation of pesticides in dung with Coleoptera parameters but not with Diptera parameters. On the two organic farms, more Coleoptera were found, whereas higher numbers of Diptera larvae and pupae were found in the dung with permethrin from Farm A. We hypothesised a negative effect on Coleoptera and Diptera, but the higher number of Dipterae larvae and pupae was unexpected. Wardhaugh et al. (1998), Sommer et al. (2001) and Gilbert et al. (2019) demonstrated a decrease in Diptera survival in dung following pyrethroid treatment of cattle. However, in the three studies, the pyrethroids deltamethrin, (alpha-)cypermethrin and cyfluthrin were used rather than permethrin. Additionally, Vale et al. (2004), who studied the effect of pour-on deltamethrin, alphacypermethrin and clyfluthrin, found that adult Diptera are less susceptible to low deltamethrin concentrations than Coleoptera and Diptera larvae; however, the percentage of hatched eggs may be lower (Mann et al., 2015). The numerically high number of Diptera larvae and pupae in dung from Farm A may be explained by a lower presence of Coleoptera larvae that predate on Diptera larvae (Sunderland, 2002; Krüger et al., 1998), thus increasing Diptera larvae survival and increasing the number of Diptera pupae, compared to treatments with fewer Coleoptera larvae. Indeed, we found a negative correlation in Experiment 1 between the number of Coleoptera on day 7 and the number of Diptera on day 14 ($r = -0.50$). Although permethrin is used against other types of flies, it looks like it indirectly increases the number of Diptera breeding and foraging on dung pats.

On Farm B, the number of different pesticides in the ration and in the dung was higher than on the other farms. As a result, the number of Coleoptera larvae were lowest in the dung from Farm B. There was also a low number of Diptera pupae in this dung, indicating a low survival rate. This may be caused by the relatively high concentration of a mixture of pesticides (cypermethrin, the synergist piperonyl butoxide, and herbicides and fungicides). As far as we know, no studies on pesticide cocktails and the effect on Coleoptera and Diptera have been performed yet.

4.4. Spiking dung with different levels of deltamethrin

To test the effect of the level of insecticides in dung on insects, we used deltamethrin to spike dung, as this is a frequently used pour-on pyrethroid. The levels used (60, 270 and 1333 μg kg^{-1} DM) correspond with levels in other studies, although for cypermethrin, Virlouvet et al. (2006) demonstrated that the concentration in dry dung can reach higher levels (up to 5000 μg kg-1 DM between the first and fourth day of the treatment). This is caused by the dose of cypermethrin applied (Virlouvet et al., 2006), which was approximately 3 times higher than the highest dose of deltamethrin used in this study. The maximum level in Experiment 2 was also somewhat lower than the permethrin concentration we observed on Farm A in Experiment 1 three days after application (sum of cis- and trans-permethrin: 1850 μg kg^{-1} DM dung). The concentration of permethrin in Tectonik (37.6 mg ml^{-1}) used on Farm A in Experiment 1 was about 5 times higher than the concentration of deltamethrin in Butox used in for spiking in Experiment 2 (7.5 mg ml^{-1}), whereas the volume of application is comparable under normal circumstances (25 ml for Tectonik®, 30 ml for Butox®).

In Experiment 2, we found that with high deltamethrin levels, the number of Coleoptera adults and the sum of Coleoptera (larvae and adults) (p-value < 0.05) were about 50% lower, whereas the number numbers of flies was about 70% higher (not significant). The lower number of Coleoptera was hypothesised; however, we had expected the differences to be more distinct. In other studies, results with comparable or higher insecticide levels also showed significant effects on mortality rate (Floate et al., 2005, 2015; Sands et al., 2018; Vale et al., 2004; Wardhaugh et al., 1998). This could be due to higher concentrations of insecticides, but also to a higher sensitivity of the invertebrates. Moreover, the higher number of Diptera larvae with higher deltamethrin concentrations was not hypothesised, as other studies showed reduced Diptera larvae or an increase in Diptera larvae mortality after treatment with pyrethroids (deltamethrin, alphacypermethrin or flumethrin; Gilbert et al., 2019; Sommer et al., 2001). However, Krüger et al. (1998) also did not observe effects of flumethrin after pour-on treatment of calves. Flumethrin has a high acaricidal activity, whereas its insecticidal properties are less pronounced (Krüger et al., 1998). The higher number of Diptera larvae in our study (in both Experiment 1 and Experiment 2) is thought to be caused by higher survival of Diptera larvae thanks to a lower predation by Coleoptera larvae. Another possible reason for the higher number of Diptera larvae on the highest deltamethrin levels is impaired development of the larvae (Vale et al., 2004; Mann et al., 2015), which would cause larvae to remain in larval stage for longer. Furthermore, some studies have shown that insects are drawn to specific pesticides (e.g. ivermectin; Römbke et al., 2010); this may also have been the case in our study and would explain the relatively high number of adult flies in the deltamethrin-treated dung. Such an attraction of insects (Diptera and Coleoptera) towards deltamethrin-treated dung is also supported by an observed trend for higher numbers of insect holes in the deltamethrin-treated dung pats on day 7 (p-value $= 0.059$; data not shown).

4.5. Ecosystem services

Contamination of dung with pesticides can have consequences for the ecosystem services provided by insects. We found that pesticides in general and more specifically permethrin and cypermethrin reduce the number of Coleoptera, which may be a factor in the disappearance of dung pats and may thus affect the food web.

Regarding the disappearance of dung pats, Coleoptera play a role in the decomposition of the dung: 10–30% of the disappearance of dung pats may be caused by Coleoptera (Holter, 1979; Svendson et al., 2003). Although the number of Coleoptera decreased by 60% after increasing the concentrations of deltamethrin, we did not measure a difference in the disappearing rate of dung pats (data not shown). An explanation could be that earthworms are more important for cow dung: up to 50% of the dung organic matter disappearance in a dung pat is caused by earthworms (Holter 1979; Bruinenberg et al., 2022). Although the number of earthworms measured was lower than in Deru et al. (2018), we did not find a negative effect of deltamethrin on earthworms in Experiment 2, which may explain why we measured no difference in dung pat disappearance between the four treatments.

Regarding the food web, the negative effects of pesticides in dung on Coleoptera may not only pose a risk to birds and the survival of the chicks who feed on Coleoptera (Wilson et al., 1999; Benton et al., 2002), but also to other taxa that feed on Coleoptera, such as bats (Catto et al., 1996; Downs and Sanderson, 2010; Ancillotto et al., 2017). Research has shown that the diets of swallow and sparrow chicks in the Netherlands contain less Coleoptera breeding and foraging on dungs pats than in neighbouring countries in Europe (Turner, 1982; Orlowski et al., 2014; Hofland, 2022). This suggests that these Coleoptera are less abundant in The Netherlands.

5. Conclusions

Based on our monitoring, it can be concluded that pesticides are widely present in feed for dairy cows in the Netherlands. Pesticides were even found in feed on organic farms, albeit with a lower incidence and concentration. A number of these pesticides in the ration (i.e. anthraquinone, chlorpropham, cypermethrin and diphenyl) are transferred to the dung of the cows, which indicates that these products are only partly degraded by the animals and that the feed containing these pesticides poses a risk to the environment. The pesticide residues from the different types of feed in the ration and from pour-on used as anti-fly treatment had a negative effect on the number of Coleoptera larvae and adults in dung. It is therefore justified to apply the precautionary principle (EU Monitor, 2000) and be aware of a possible contamination of animal feed with different types of pesticides and of the potential risk to the environment and ecosystem services. More frequent analysis of pesticides in animal feed and dung, as well as publicly sharing these data will further outline the risk of this contamination to the survival of dung-bound invertebrates and to the birds and other animals, such as bats, who feed on these species.

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.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2022.108307.

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