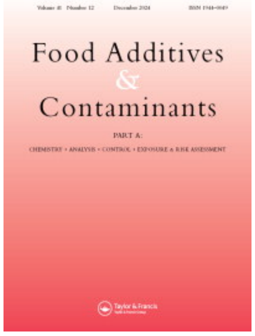

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Sustainable dairy farming and fipronil risk in circular feeds: insights from an Italian case study

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ABSTRACT

Circular feeds, such as grain dry distillers, citrus pulp, cane molasses, and potatoes peels, are co-products of biomass processes. They are currently proposed in animal nutrition to improve the environmental and economic sustainability of the food production chain. In this paper, we report a case study involving fipronil, a pesticide currently not authorized for agriculture within the EU, but used in the Americas, Eastern Europe, and Asia. Fipronil was found at a mean level of 0.49 mg/kg, in a grain dry distiller batch administered to dairy cows. This finding, along with other evidence of potential fipronil presence in feed materials, prompted us to evaluate the risk to food safety and food security from 12 different conventional and sustainable feeding regimens. To this purpose, we considered a fipronil feed-to-milk carry-over rate of 0.52, the tolerance levels in fodders and food from The EU, Codex Alimentarius, and US-EPA, and the Acceptable Daily Intake (ADI) of 0.0002 mg/kg body weight for adverse effects on thyroid function in dairy cows. Under a conservative scenario, fipronil-contaminated potato peels and grain distillers in the feeding regimens may play a pivotal role in exceeding the EU Maximum Residue Level (MRL) in bovine milk and fat (0.005 and 0.030 mg/kg, respectively). Hay-based diets with soybean hulls and cane molasses show negligible risks (Hazard Index ~ 1). In all cases, the ADI exceedance suggests the need to evaluate thyroid function in dairy cows exposed to fipronil as a food security factor.

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Exposure assessment; LC/MS; risk assessment – modelling; pesticides; feeding; residues; animal feed; milk; oils; fats

Introduction

In recent years, consumers have become increasingly aware of social and environmental issues, leading to a growing demand for more sustainable animal products. As a result, various production sectors, including dairy farming, are moving towards a more rational use of natural resources, especially soil and water, to help meet these demands (FAO, 2018).

The most impactful strategy for farmers has been optimizing animal nutrition to improve production while also reducing the environmental impact of dairy farming. Innovative nutritional strategies include using formulations with raw materials that are suitable for local cultivation and incorporating feed additives that enhance the digestibility and conversion efficiency of feed into milk (Cavallini et al. 2021; NASEM 2021). Recently, taking a bioeconomy approach, the European Feed Manufacturers' Federation (FEFAC 2022) has promoted the recovery of secondary raw feed materials like grain-derived distillers, sugarcane molasses, and potato peel. This initiative introduces a broad array of ingredients known as 'agro-industrial co-products' or 'circular feeds,' which have a low environmental footprint. Beyond reducing greenhouse gas (GHG) emissions associated with feed production, the adoption of these circular feeds has been suggested as a resilience response to shifting feed and food

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security dynamics triggered by the Russian invasion of Ukraine in February 2022 (Leal Filho et al. 2023). According to the European Parliamentary Research Service (EPRS 2022), this geopolitical event compromised the EU Open Strategic feed autonomy: Ukraine normally supplies almost half of the cereals (52% of EU maize imports), most addressed to feed production.

In this context, the commercial flow of oil, grain and other vegetables that could serve as sources for the feed industry's recovery 'circular feeds' (Rabobank 2021) involves countries with varying legislation regarding pesticide use in agriculture, such as fipronil (Braga et al. 2020).

'Fipronil (5-amino-1-[2,6-dicloro-4-(trifluorometil)fenil]-4-[(trifluorometil)sulfinil]-1H-pirazol-3-carbonitrila), is a highly active, systemic and broad spectrum insecticide used to control ants, beetles, cockroaches, fleas, ticks, termites, mole crickets, thrips, rootworms, weevils, and other insects. For its high insecticidal activity, environmental persistence (Fipronil is ranked among the per- and poly-fluorinated substances – PFAS 'forever chemicals' class – as matter of the presence of CF_3 moieties in its chemical structure), and low toxicity for mammalians, is still widely used in pest control in agriculture and urban settlements and used in vector control treatments for pets as over-the counter veterinary drug (Singh et al. 2021). The half-life of fipronil is 122–128 days in aerobic soils. Under aerobic conditions, naturally occurring soil organisms break down fipronil to form fipronil-sulfone. Fipronil can also be hydrolysed to form fipronil-amide. Fipronil degrades rapidly in water when exposed to UV light to form fipronil-desulfinyl. Under these conditions, fipronil has a half-life of 4 to 12 h (https://echa.europa.eu/it/brief-profile/-/briefprofile/100.102.312). Due the eco-toxicity of the parent compound and its metabolites and degradation products to pollinating insects like honeybees (PAN 2012), its use in agriculture has been prohibited in the EU since 2014, due to the lack of request about the renewal authorization from applicants (European Commission Delegated Regulation EU, 2019), while it still in place for non-agriculture use under the EU Reach Regulation (EU) No 528/2012 as biocidal product-type 18, and for pets treatment as veterinary drug licensed by the European Medical Agency (Gupta and Anadón 2018). These non-agriculture uses are a matter of an increasing eco-toxicological concern for aquatic organisms, living in fresh and transitional waters in vicinity of urban wastewater effluents from treatment plants (Wu et al. 2015; Mamboungou et al. 2024), where the weight of the contribution from the non-agriculture/biocidal use and the pet treatment has to be deepened (Perkins et al. 2024). In mammals, toxicological concerns focus on thyroid function and associated neurodevelopment impacts caused by both the parent compound and its sulfone metabolite, with an acceptable daily intake (ADI) of 0.0002 mg/kg (EFSA 2023) prompted the EU to ban its use in food producing animals.

Internationally, there is no consensus on restricting the use of fipronil to agricultural premises, and there is a lack of agreement on the definition of fipronil residues. In the EU the definition of the residue for compliance with MRL in plant and animal commodities, as well as for dietary intake assessment, accounts for the sum of fipronil and 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfonyl pyrazole (MB46136) expressed as fipronil, with an MRL of 0.005 mg/kg in milk and 0.030 mg/kg in fat (European Commission Regulation 2024). According to the Codex Alimentarius (2021), the residue definition for intake assessment includes two other metabolites/degradation products: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethy lphenyl)-4-trifluoromethylthiopyrazole (MB45950) and 5-amino-3-cyano-1-(2,6- dichloro-4-trifluoromethylphenyl)-4-trifluoromethylpyrazole (MB46513), with an MRL of 0.020 mg/kg for both dairy milk and fat. The US-EPA in its technical note 40 CFR 180.517 (2024) includes all fipronil residues (fipronil and the sulfone, sulfinyl, and desulfinyl forms) for compliance with maximum residue levels in plant and animal commodities (Table 1 and Figure 1; SM). It is worth noting that both the EU and Codex Alimentarius recognise the aforementioned ADI as a health-based guidance value for chronic dietary exposure.

A recent opinion on the presence of fipronil in molasses from sugarcane and potato peel (EFSA 2023), along with an Italian case study involving distiller contamination in dairy cow nutrition

Table 1. Regulatory levels (mg/kg) for fipronil residues in selected food and feed commodities, according to Codex Alimentarius, European Commission and US-EPA legislative frameworks.

Commodity	Codex (a)	EU (b) Reg 2024/347	US EPA (c) 40 CFR 180.517
Wheat	0.002	0.005	0.002*
Wheat, Forage			0.020*
Wheat, hay			0.030*
Wheat, straw			0.030*
Sunflower seed	0.002	0.005	
Rice, hay and/or straw	0.200$		
Potato	0.020	0.005	0.030
Potato peels, wet			0.100
Oats	0.002	0.005	
Maize fodder	0.100$		
Maize	0.010	0.005	0.020
Maize stover			0.300
Maize, forage			0.150
Barley	0.002	0.005	
Sugarcane/molasses		0.010	
Cattle milk	0.020	0.005	1.50§ 0.050
Cattle meat	0.500§	0.005	0.040
Cattle liver	0.100	0.005	0.100
Cattle kidney	0.020	0.005	0.040
Cattle fat	0.020	0.030	0.400

[a]Definition of the residue for compliance with the MRL for plant and animal commodities: Sum of fipronil and 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4- trifluoromethylsulfonylpyrazole (MB46136; sulfone) expressed in terms of fipronil. Definition of the residue for dietary risk assessment for plant and animal commodities: Sum of fipronil and 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4- trifluoromethylsulfonylpyrazole (MB46136; sulfone), 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylthiopyrazole (MB45950; sulfide) and 5-amino-3-cyano-1-(2,6- dichloro-4-trifluoromethylphenyl)-4-trifluoromethylpyrazole (MB46513; desulfinyl) expressed in terms of fipronil. The residue is fat-soluble.

[b]Definition of the residue for compliance with the MRL for plant and animal commodities: sum fipronil + sulfone metabolite (MB46136) expressed as fipronil

[c]Definition of the residue for compliance with the MRL for plant and animal commodities: Fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-[(1R,S)-(trifluoromethyl)sulfinyl]-1Hpyrazole-3-carbonitrile) and its metabolites 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl) sulfonyl]-1H-pyrazole-3-carbonitrile (MB46136; sulfone) and 5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-[(trifluoromethyl) thio]-1H-pyrazole-3-carbonitrile (MB45950; sulfide)and its photodegradate 5-amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1R,S)-(trifluoromethyl)]-1Hpyrazole-3-carbonitrile (MB46513; desulfinyl).

*Inadvertent residues.

$Dry weight base.

§Lipid base.

(Italian Ministry of Health 2024), prompted us to investigate the potential combined exposure of dairy cows to various feed materials contaminated with fipronil. This study aims to assess the risk to food security, including the compromise of animal welfare and reduced milk production. Furthermore, we consider food safety concerns, specifically the potential to exceed EU regulatory limits (European Commission Regulation 2024), due to the inadvertent presence of fipronil in feed materials within a global market scenario, selected under a 'circular feed economy' appraisal.

Materials and methods

The Italian case study

In November 2023, during routine monitoring activities for plant protection products and biocides used in livestock farming, a sample of adipose tissue taken from a dairy cow at a slaughterhouse was found to exceed the EU regulatory limit of 0.005 mg/kg for fipronil, as specified in European Commission Reg. 2019/1792. Follow-up activities at the farm identified complementary feed as the source of the fipronil contamination. Further investigations into the various ingredients in feed traced the primary source of contamination to dried distiller grains, which were still available in sealed bags. Identification and quantification of fipronil was based on the residue definition set in Regulation (EU) 396/2005, which includes the sum of fipronil and its sulfone metabolite (MB46136), expressed as fipronil. An accredited method, compliant with the European Commission, SANTE 11312/2021 (European Commission, SANTE 2022) guidance, based on LC-MS/MS technique, was used to analyze compound feed and related feed materials, dried distillers grain included. The analytical method is described in the supplementary materials and the results of this investigation on contaminated distillers grains are reported in Table 2. Residues of metabolite MB46136 were expressed as 'fipronil' by applying the molecular weight conversion factor of 0.965 (EFSA 2023).

Fipronil in forages and circular feeds

The figures regarding fipronil concentration (as the sum of the parent compound and the sulfone metabolite MB46136) in forages and feeds were derived from the following sources: a) the EFSA opinion (EFSA 2023) on sugarcane molasses imported from Brazil; b) the tolerance levels specified in the US Code of Federal Regulations (US EPA 2024) for wet potato peel and corn forages and grain (voluntary use); and c) contamination levels found in cereal-derived distillers from the Italian case study reported below. For other circular feeds originating from EU imports of commodities that might be subject to fipronil use, such as citrus pulp and soybean hulls, we

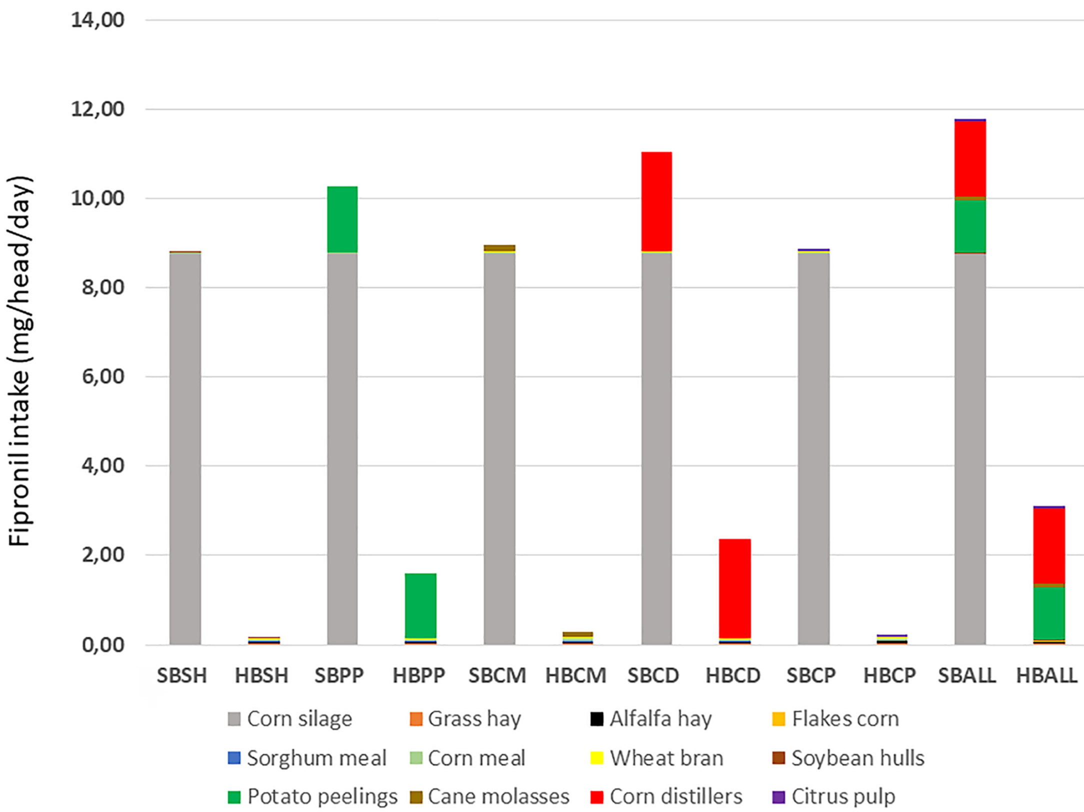

Figure 1. Fipronil intake (mg/head/day) from the different feed materials in dairy cows, according to the modelled feed ratios and the contamination scenario considered. SBSH: silage-based diet with soybean hulls; HBSH: hay-based diet with soybean hulls; SBPP: silage-based diet with potato peelings; HBPP: hay-based diet with potato peelings; SBCM: silage-based diet with cane molasses; HBCM: hay-based diet with cane molasses; SBCD: silage-based diet with corn distillers; HBCD: hay-based diet with corn distillers; SBCP: silage-based diet with citrus pulp; HBCP: hay-based diet with citrus pulp; SBALL: silage-based diet with the combined presence of all by-products; HBALL: hay-based diet with the combined presence of all these by-products.

consider the 0.005 mg/kg limit (12% moisture) specified in the EU food legislation (European Commission Regulation 2024), due to the lack of monitoring data. All considered contamination values were converted and expressed on a dry matter basis as reported in Table S1 of the supplementary materials.

Simulation of experimental rations

Theoretical dairy cows feeding rations were designed using NDS Pro software (version 6.5, RUM&N Sas RE, Italy) based on the Cornell Net Carbohydrate and Protein System (CNCPS) model (Buonaiuto et al. 2021). This dynamic rationing software was utilized to formulate twelve different rations, as detailed in Table 3. These formulations align with the NASEM recommendation (NASEM 2021) to assess the impact of various industrial byproducts on pesticide intake in dairy cow diets. To replicate the realistic feeding practices prevalent in northern Italy, we created six hay-based (HB; Cavallini et al. 2023) rations and six silage-based (SB; Felini et al. 2024) rations.

The simulations targeted a typical Italian Holstein dairy cow, averaging 650 kg in body weight, 150 days in milk (DIM), 50 days of gestation, and 30 kg/day of milk containing 4% fat, 3.45% protein, and 4.85% lactose. The simulated housing conditions mirrored the average environmental parameters of dairy farming in Northeast Italy, featuring night-time temperatures of +5 °C, day-time temperatures of +8 °C, 70% relative humidity, daily animal activity of 500 min, approximately 12 h of rest per day, and an average of 7 meals per day.

The base components of each ration included hay and cereals supplemented with five different industrial byproducts: soybean hulls (SH), potato peelings (PP), cane molasses (CM), corn distillers (CD), and citrus pulp (CP). Furthermore, one ratio, labeled 'ALL,' was formulated to simulate the combined presence of all these by-products, providing a comprehensive scenario. Each ingredient and byproduct was selected based on its known composition and relevance to regional feeding practices. The inclusion levels were determined by the typical usage rates observed in local farming operations, ensuring that the simulations accurately reflected real-world scenarios. The considered formulations are reported in Table 3.

Table 2. Results of independent determination of Fipronil and its main sulfone metabolite (MB46136) residues, according to EU legislation, at farms from unpackaged and packaged bags of complementary feed–grain dry distiller. Values expressed in mg/kg.

Unpackaged bags	FIPRONIL	FIPRONIL MB46136*	∑ FIPRONIL
1	0.55	0.017	0.57
2	0.38	0.014	0.39
3	0.30	0.012	0.31
4	0.35	0.014	0.36
5	0.33	0.013	0.34
6	0.52	0.016	0.54
7	0.33	0.013	0.34
8	0.34	0.013	0.35
9	0.86	0.030	0.89
10	0.51	0.017	0.53
11	0.38	0.014	0.39
12	0.55	0.019	0.57
13	0.43	0.015	0.45
14	0.93	0.033	0.96
15	1.60	0.049	1.7
16	0.37	0.014	0.38
17	0.42	0.015	0.44
18	0.62	0.020	0.64
19	0.59	0.018	0.61
20	0.47	0.015	0.49
Mean	0.54	0.019	0.56
CV%	54	48	55
Packaged bags	**FIPRONIL**	**FIPRONIL MB46136***	**∑ FIPRONIL**
1	0.47	0.015	0.49
2	0.5	0.016	0.52
3	0.54	0.017	0.56
4	0.37	0.014	0.38
5	0.50	0.017	0.52
6	0.24	0.008	0.25
7	0.46	0.014	0.47
8	0.52	0.015	0.54
9	0.64	0.021	0.66
mean	0.47	0.015	0.49
CV%	22		

*Converted as Fipronil residue applying a molecular weight factor of 0.965.
CV: coefficient of variation

Modeled fipronil intake scenario and risk assessment of food security and food safety

According to the modeled fipronil occurrence and the considered feed ratios, we derived intake estimates that were compared with the ADI of 0.0002 mg/kg, to compute the Hazard Index (HI), which is the ratio between the estimated intake and the maximum acceptable intake (EFSA 2024). This HI can be referred to the food security assessment related to thyroid gland disruption, which serves as a proxy for reduced milk yield (Fazio et al. 2022).

For food safety, we considered the following regulatory values: a) the MRL of 0.030 mg/kg in bovine fat and 0.005 mg/kg in milk (European Commission Regulation 2024); b) the MRL of 0.020 mg/kg in bovine milk from the Codex Alimentarius (2021); and c) the US EPA MRL of 0.050 mg/kg in bovine milk (Table 1). To this end, a feed-to-milk carry-over rate of 0.52 was derived from the literature (Le Faouder et al. 2007), referred to $n = 12$ dairy cows exposed to a naturally contaminated silage, for 90 days. This rate has been recovered from the mean of 6 different paired intake and milk excretion data as

Table 3. Ingredients (kg/head/day) and cost of the ration (€/head/day) according to the Italian institute for food and agriculture services quotation (ISMEA 2023) for the considered experimental rations[2].

	Corn silage	Grass hay	Alfalfa hay	Flakes corn	Sorghum meal	Corn meal	Wheat bran	Soybean hulls	Potato peelings	Cane molasses	Corn distillers	Citrus pulp	Min-Vit supplement	Feed cost
SBSH	35	0	0	0	2	2	2	5	0	0	0	0	0.8	4.81
HBSH	0	6	5	0	4	3	2	5	0	0	0	0	0.8	5.82
SBPP	35	0	0	0	2	2	2	0	5	0	0	0	0.8	3.72
HBPP	0	6	5	0	4	3	2	0	5	0	0	0	0.8	4.73
SBCM	35	0	0	0	2	3	5	0	0	1.5	0	0	0.8	4.81
HBCM	0	6	5	0	4	4	5	0	0	1.5	0	0	0.8	5.83
SBCD	35	0	0	0	1	3	4	0	0	0	4	0	0.8	5.06
HBCD	0	6	5	0	3	4	4	0	0	0	4	0	0.8	5.15
SBCP	35	0	0	0	1	3	4	0	0	0	0	10	0.8	4.36
HBCP	0	6	6	0	3	4	4	0	0	0	0	10	0.8	5.63
SBALL	35	0	0	0	0	0	0	4	4	1	3	9	0.5	4.65
HBALL	0	6	5	2	0	0	0	4	4	1	3	9	0.5	5.52

[1]SBSH: Silage-based diet with soybean hulls; HBSH: Hay-based diet with soybean hulls; SBPP: Silage-based diet with potato peelings; HBPP: Hay-based diet with potato peelings; SBCM: Silage-based diet with cane molasses; HBCM: Hay-based diet with cane molasses; SBCD: Silage-based diet with corn distillers; HBCD: Hay-based diet with corn distillers; SBCP: Silage-based diet with citrus pulp; HBCP: Hay-based diet with citrus pulp; SBALL: Silage-based diet with the combined presence of all by-products; HBALL: Hay-based diet with the combined presence of all these by-products.

pool, over the exposure time, under the assumption of a steady state. At steady state, the fipronil concentration in milk, computed on fat basis, is considered in equilibrium with that of adipose tissue. For food safety, we performed a preliminary risk characterization based on the HI, computed as the ratio between the modeled Fipronil concentration in selected food commodities and the pertinent regulatory limits (Table 1).

Based on the proposed rations shown in Table 3, we determined the maximum tolerable fipronil intakes from each feed material to prevent residues non-compliance in milk, according to the EU legislation (Table 1).

Results and discussion

Dairy cow's intake estimates and risk characterization

Conservative estimates of fipronil intake by dairy cows *via* different sustainable feed formulas are shown in Figure 1. Figure 2 reports the HI for food safety, considering the 0.52 feed-to-milk carry-over rate and the different regulatory frameworks. Figure 3 illustrates the HI for animal welfare (food security), based on the ADI of 0.0002 mg/kg for adverse effects on thyroid gland. In Italy, the need to import feed materials from non-EU countries exposes farmers to the risk of fipronil contamination, due to differing legislation. This risk was highlighted in our study, which focused on grain distillers, and was also addressed by the EFSA (2023) in their assessment of cane molasses from Brazil. Therefore, the presence of fipronil residues from various feed materials cannot be overlooked within the global market and import flows from regions where pesticide regulations differ from those in the EU.

Regarding the Italian case study, results from Table 2 clearly show a rather homogeneous distribution of fipronil residues in packaged dry distillers grain bags. This supports the conclusion of a consistent presence in the original fodder, indicating systemic agricultural use rather than occasional post-harvesting contamination or unauthorized farm use. Differences noted between packaged and unpackaged samples (means 0.49 vs 0.56 mg/kg product, respectively; Table 2) may

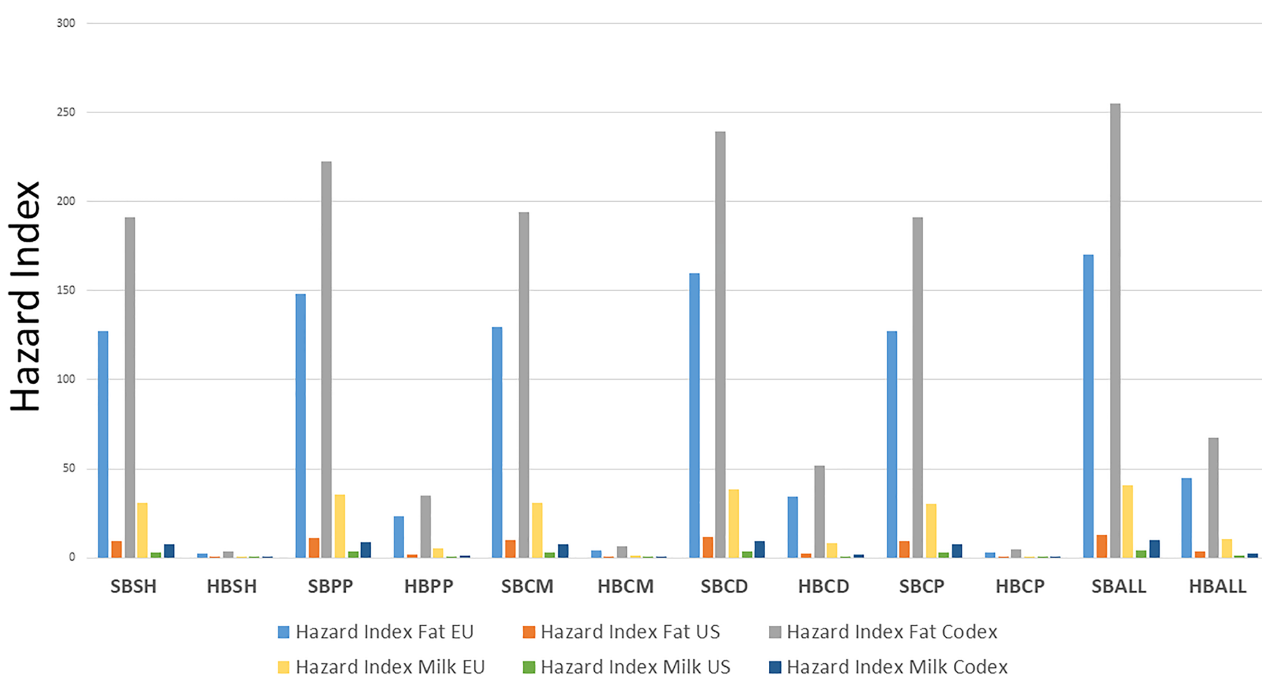

Figure 2. Food safety related HI of the modelled fipronil intake in dairy cows for the different feed regimens considered, accounting for the residue limits in dairy milk according to the European Union Regulation (2024) the Codex Alimentarius, and the US-EPA (see Table 1). SBSH: silage-based diet with soybean hulls; HBSH: hay-based diet with soybean hulls; SBPP: silage-based diet with potato peelings; HBPP: hay-based diet with potato peelings; SBCM: silage-based diet with cane molasses; HBCM: hay-based diet with cane molasses; SBCD: silage-based diet with corn distillers; HBCD: hay-based diet with corn distillers; SBCP: silage-based diet with citrus pulp; HBCP: hay-based diet with citrus pulp; SBALL: silage-based diet with the combined presence of all by-products; HBALL: hay-based diet with the combined presence of all these by-products.

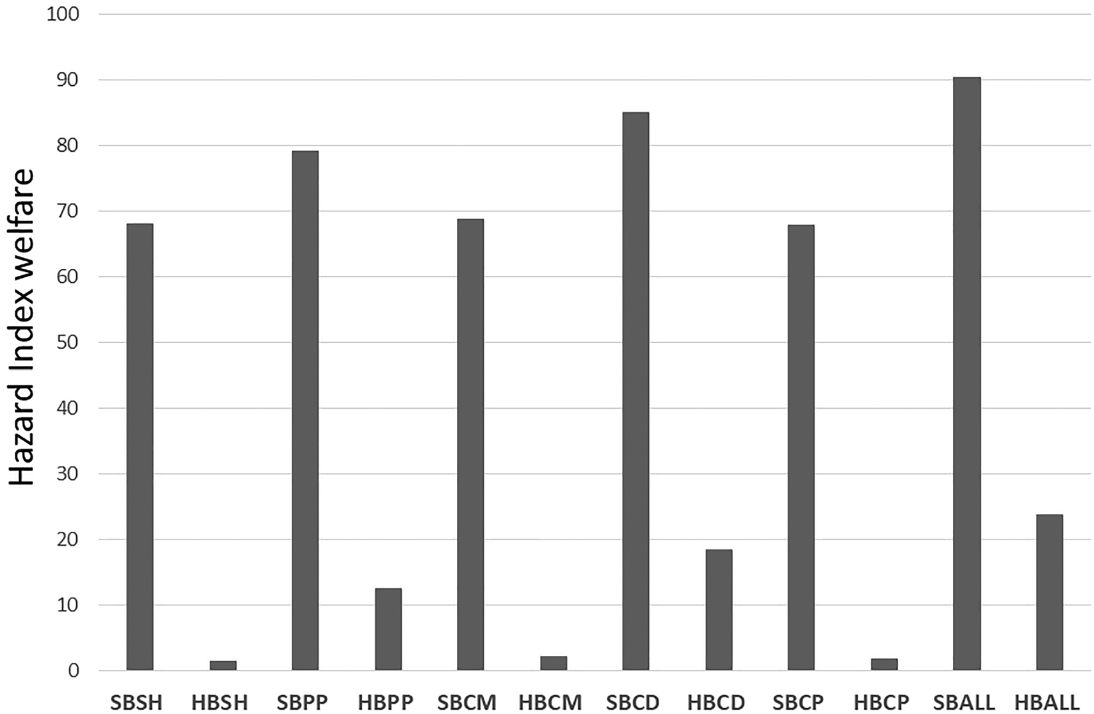

Figure 3. Food security related HI of the modelled fipronil intake in dairy cows for the different feed regimens considered, accounting for the ADI of 0.0002 mg/kg for adverse effects on thyroid gland. SBSH: Silage-based diet with soybean hulls; HBSH: hay-based diet with soybean hulls; SBPP: silage-based diet with potato peelings; HBPP: hay-based diet with potato peelings; SBCM: silage-based diet with cane molasses; HBCM: hay-based diet with cane molasses; SBCD: silage-based diet with corn distillers; HBCD: hay-based diet with corn distillers; SBCP: silage-based diet with citrus pulp; HBCP: hay-based diet with citrus pulp; SBALL: silage-based diet with the combined presence of all by-products; HBALL: hay-based diet with the combined presence of all these by-products.

reasonably be attributed to the analysis of different batches used on farms. The desulfynil form (MB46513), not covered by EU pesticide residue legislation, was not reported. Follow-up investigations are in progress to determine the geographical provenience of the lots.

Country-based scenarios

In Italy, dairy cow farmers are largely self-sufficient in corn silage production. Therefore, the intake scenarios for silage-based diets SBSH, SBPP, SBCD, SBCP, and SBALL (Figure 1) may not accurately reflect Italian conditions due to the EU ban on fipronil in agriculture (see Figure S1). In countries where fipronil is authorized in *Zea mays* cultivation, the computed silage-based intakes could impact animal welfare and lead to fipronil residues in milk that exceed the EU MRL by 40–180 times (Figure 2), assuming chronic and regular intake leading to a steady-state condition in the herd. This also highlights a potential risk for animal products imported from non-EU Countries.

In milk, according to the Codex Alimentarius and US-EPA MRLs (Table 1), the increase in the HI is less evident compared to the EU, indicating a better alignment between tolerable feed contamination levels and regulatory residue limits in milk. Conversely, the EU's MRL of 0.005 mg/kg leads to a higher risk of non-compliance. For fipronil residues in fat, the HI clearly indicates a risk of non-compliance under EU and Codex Alimentarius standards, while US legislation appears more consistent with US-EPA tolerated residues in feeds. In the Supplementary Materials (Figure S2), we provide a HI scenario for the EU, assuming fipronil contamination from imported circular feeds, only (see Figure S1).

The intensive and non-rotating cultivation of *Zea mays*, from which silage is derived, is now considered an unsustainable agricultural practice due to its significant water footprint and the ecological risk posed by mycotoxin-producing fungi.

Consequently, it is important to consider how substituting corn silage with hay from permanent grasslands might affect fipronil contamination and HI profiles (Figures 1–3). Additionally, Figure S1 illustrates the contribution to fipronil intake from imported circular feeds in the EU, assuming baseline contamination levels for silage and hay. Among 'circular feeds' or 'feed co-products,' PP and CD play a pivotal role compared to CM, highlighted by EFSA in its 2023 opinion. Under the proposed conservative scenario, ranked by decreasing HI order, HBALL (a hay-based diet with all these co-products), HBCD (a hay-based diet with corn distillers), and HBPP (a hay-based diet with potato peelings) present risk profiles for food security and safety, particularly concerning EU MRLs in milk and fat. In contrast, HBSH (a hay-based diet with soybean hulls), HBCM (a hay-based diet with cane molasses) and HBCP (a hay-based diet with citrus pulp) exhibit negligible risks. Regarding animal welfare and food security, HB rations show the lowest HIs. Within this context, imported CD and PP significantly contribute to over-exposure compared to other circular feed materials (CP and CM). It is however worth noting that according to Global Import data, during 2023 orange pulp import shipments from Brazil (where fipronil use is allowed) stood at 1.6K tons, imported by 160 World Importers from 55 Brazil Suppliers (https://www.volza.com/p/oranges-pulp/import/coo-brazil/); in the past, CP imports from Brazil were the cause of intolerable levels of 'dioxins' in dairy products from Germany (De Lacerda 2019). In Table S6, we provide a snapshot of the top supplier and top import Countries of circular, useful for a risk orientation on the geographical origin of the products.

Safety and sustainability; the economic drivers

It is worth noting that feed costs represent around 50% of a dairy operation's total expenses, with half of these costs attributed to feed imports (ISMEA 2023). The persistent rise in production costs necessitates that farmers implement increasingly effective strategies to enhance farm profitability, particularly by addressing the significant expense of cow feed (Buonaiuto et al. 2021). A crucial aspect of this effort involves examining sourcing methods and evaluating feed ingredients, whether forage or concentrates. One widely adopted strategy among farmers is the use and valorization of by-products from other industries. When used appropriately, these by-products can maintain optimal performance while reducing feed costs. Generally, these products are more affordable than concentrates, allowing for a reduction in feed ration costs without compromising productivity, thereby safeguarding the farm's financial stability (CLAL 2023).

Within this context, a comparison of the fipronil contribution to the intake from circular feeds (Figure 1 and Figure S1) and the ration costs (Table 3) shows that using potato peel in SBPP, SBALL, and HBPP formulas (3.75, 4.65, and 4.73€/head/day, respectively) can provide an economic benefit for farmers, offering cost savings from 19% to 36% while balancing food safety and security risks. This economic advantage, although to a lesser extent, also applies to grain distillers, with cost savings ranging from 5.2% to 13%. Considering fluctuations in global market prices, monitoring feed material prices along with the traceability of circular feeds serves as a useful tool for risk-oriented internal and official checks (European Commission, 2020).

Uncertainties/limitations of this study

Studies on the occurrence of fipronil in selected feed materials are sparse (EFSA 2023), and do not always extend to all potential metabolites and degradation products. Moreover, proposed feed consumption databases (Pinotti et al. 2024), do not yet encompass all the proposed circular feeds. The results from the reported case-study, lacking a consolidated database about fipronil occurrence in imported feeds, do not allow a statistical elaboration of the data, in terms of percentiles of occurrence. Additionally, we did not consider fipronil residue intake from freshwater sources impacted by residential use, as this insecticide is not used in agriculture (Wu et al. 2015). Uncertainties exist in both directions of under- and over-estimation of intake.

Under a conservative scenario, we considered the possibility of fipronil simultaneous presence in the same feeding ration from different materials. In future, with results from regular, risk-oriented monitoring plans and real-time updated feed consumption databased (Pinotti et al. 2024), we could provide a refined probabilistic assessment. This could address the potential over-estimation of intakes in rations using various circular co-products, as seen in the case of SBALL and HBALL diets (Table 3 and Figure S2).

The effects of fipronil on the thyroid gland of dairy animals have been described, with one report indicating sheep are less sensitive to its toxicological effects than other mammals (Leghait et al. 2010). This uncertainty suggests a lower risk concerning food security. Owing to the above, it seems reasonable to consider a HI > 10 for risk characterization in ruminants (Figure 2; Figure S2).

Feed-to-milk carry-over rates

Experimental studies on fipronil toxicodynamics/toxicokinetics in lactating dairy cows have been provided by applicants and reviewed by EFSA in its 2006, 2012, and 2023 opinions, in order to propose MRLs in feed and food. The first study framed the exposure at three dose levels of 0.0011, 0.0031, and 0.0101 mg/kg bw day for 35 consecutive days, indicating residues observed in milk and animal tissues strictly linear to the dose levels in animal feed (EFSA 2006). In the second study, dairy cows were administered fipronil for 20 days at a rate equivalent to 0.0402 mg/kg bw day, indicating fipronil residues reach a plateau slowly (EFSA 2006, 2012). A more recent feeding study presented to EFSA by an applicant and evaluated in EFSA (2023) opinion was based on fipronil once daily at dose rates of 0.00011 and 0.0011 mg/kg bw day for 50 consecutive days. In milk, residues of fipronil, at the higher dose level, were found in milk only after day 35 of dosing, with plateau 35–40 days after the start of the dosing. The depuration data indicate that residues do not reach levels below LOQ even on day 70 (0.001 mg/kg). The study has been performed according to requirements of the OECD guidelines on residues in livestock (OECD 2007).

In the present paper, we preferred to consider as benchmark the study by Le Fadouer et al. (2007), to derive the feed-to-milk carry-over rate, for the following reasons. The data and metadata, well supported from methods validation, are fully available via open access peer reviewed publication and produced by an independent group; the data are referred to a 90 day long exposed dairy cows ($N = 12$ vs $N \geq 3$ × dose level, requested by OECD 2007), with a reported intake in the range of 0.007 - 0.008 mg/kg/head per day; data are referred to naturally contaminated silage, and, for this reason, closer to the Italian case-study from the field. The already assessed strict linearity of fipronil residues in milk and animal tissues to the dose levels in animal feeds makes the data from French study and the derived carry-over rate robust and eligible for consideration for field exposure under our proposed scenario, with estimated intakes from <0.001 mg/kg bw per day for HBSH, HBCM, and HBCP rations, through >0.001 < 0.005 mg/kg bw per day of HBPP, HBCD, and HBALL rations, up to the 0.0136–0.0181 mg/kg bw per day from SBSH, SBPP, SBCM, SBCD, SBCP, and SBALL. Occasional and short-term exposures should account for a lesser extent of carry-over. Fluctuations in feed material cost and related changes of the geographical origin may alter exposure scenarios.

Conclusions

Due to the extensive use and environmental persistence of fipronil, residues of this insecticide can be expected to be present in different feed materials, depending on their geographical origin. This possibility should be considered when setting import tolerances. From the case-study reported in this work, we drafted circular feeds-based exposure scenarios in dairy cattle that do not exclude effects on animal welfare and food security, as well an increase of fipronil residues occurrence at levels of potential relevance both for the compliance with the food safety regulation, and for the Consumer's intake assessment. The proposed case-study suggests the assessment of proposed import tolerances in feeds should be supported from datasets coming from regular and risk-oriented monitoring activities. To this purpose, the presence of differing regulations and residue limits in foods, not always expressed on a fat basis despite fipronil lipophilicity, may require a re-evaluation of MRLs on fat basis. Furthermore, there is an ongoing concern regarding the environmental sustainability of feed co-products contaminated with fipronil, given its well-documented ecotoxicity for aquatic species and pollinators.

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The authors do not have any conflicts of interest to declare.

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Author contributions

Gianfranco Brambilla: Conceptualization, Validation, Writing- Original draft preparation. Mara Gasperini: Methodology, Investigation, Resources. Simonetta Menotta: Methodology, Investigation, Resources. Giovanni Albrici: Supervision, Resources, Visualization. Valeriano Avezzù: Investigation. Roberta Vitali: Investigation. Giovanni Buonaiuto: Writing- Original draft preparation, Writing- Reviewing and Editing. Martina Lamanna: Visualization, Writing- Reviewing and Editing. Damiano Cavallini: Conceptualization, Validation, Writing- Original draft preparation.

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Data availability statement

The data used in the current study are available upon request from the corresponding author.

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