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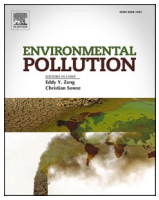

Pesticides residues in pet food: A market-based study on prevalence and toxicological implications

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ABSTRACT

Pesticide residues in pet food pose potential risks to animal health, yet their occurrence and dietary exposure in companion animals remain largely unexplored. This study analyzed 83 commercial dry pet food products (43 for dogs and 40 for cats) from the Spanish market to assess pesticide contamination and associated toxicological risks. A total of 51 pesticides were detected, predominantly fungicides (47 %) and insecticides (37 %), with 37.25 % of them banned in the European Union. Pesticide residues were significantly more prevalent in pet food containing vegetable ingredients ($p = 0.041$). Although pesticide residues were detected more frequently in dog food than in cat food ($p < 0.05$), total pesticide concentrations did not significantly differ between species. The estimated daily intake (EDI), calculated according to manufacturer-recommended feeding rates, revealed significant differences in exposure levels between dogs and cats for specific compounds. However, cumulative exposure assessments through the Hazard Index (HI) indicated that all pesticide groups remained below the risk threshold ($HI < 1$), with a worst-case scenario of 0.32. Despite the frequent detection of non-approved pesticides and regulatory concerns, our findings indicate that chronic dietary exposure to these pesticide residues in pet food is unlikely to pose an immediate toxicological risk, based on calculations using current regulatory thresholds, which are established for individual compounds. However, the long-term effects of chronic low-dose exposure to pesticide mixtures remain uncertain and require further investigation. The absence of specific maximum residue limits (MRLs) for pet food underscores the need for stricter regulations and systematic monitoring to ensure long-term safety. To our knowledge, this is one of the first comprehensive investigations assessing both pesticide prevalence and potential dietary exposure in companion animals, highlighting the urgent need for improved regulatory frameworks to address the presence of non-approved pesticides in pet food.

1. Introduction

The pet food industry has grown substantially in recent years, driven by the increasing number of companion animals and the rising awareness of pet nutrition among owners. According to the European Pet Food Federation (FEDIAF), Europe's pet and cat population reached 235 million in 2022, comprising 129 million cats and 106 million dogs (FEDIAF, 2025a). The pet food market generated €29.2 billion annually, with a production volume of approximately 10.5 million tonnes (FEDIAF, 2025b; 2025a). This sector directly employs around 283,000 people and indirectly supports an estimated 2.3 million jobs through related services and products. This growth trend is consistent across the European Union, reflecting changes in household spending patterns. In Spain, the industry has shown consistent growth, with a turnover of €1.955 billion in 2023, a 14.5 % increase from €1.708 billion in 2022 (ANFAAC, 2025). This upward trend underscores the growing importance of pet ownership and the increasing demand for high-quality pet nutrition. Spain's pet population includes approximately 9.3 million dogs and 5.9 million cats. Dry food remains the dominant segment, representing 88.1 % of dog food sales and 74.8 % of cat food sales (ANFAAC, 2025). This market expansion underscores the need for continuous monitoring of pet food safety and quality.

Modern pet food formulations contain a diverse range of plant-based ingredients, such as cereals (e.g., corn, rice, wheat, barley, and sorghum)

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and various vegetables, providing energy, protein, fiber, and essential micronutrients (FEDIAF, 2024). While cereals have traditionally been the primary carbohydrate source in pet food, there is a growing trend toward incorporating vegetables —such as peas, potatoes, carrots, legumes— driven by their perceived health benefits and the rising popularity of grain-free diets (FEDIAF, 2019; Vinassa et al., 2020). Beyond serving as an energy source, plant-based ingredients contribute dietary fiber, which supports gut microbiota and improving stool quality (Tanprasertsuk et al., 2022). Moreover, plant-derived phytonutrients, —such as polyphenols, carotenoids, and flavonoids— possess antioxidant, anti-inflammatory, and immune-supporting properties, further enhancing their appeal in commercial pet food formulations (Guo et al., 2024; Tanprasertsuk et al., 2022).

Nevertheless, the increasing use of plant-based ingredients in pet food formulations has raised concerns about the presence of contaminants, particularly pesticide residues. Many pesticides applied during the cultivation, transportation, and storage of raw materials persist in the final pet food product (Kumar et al., 2020; Na et al., 2022). Additionally, animal-derived ingredients contribute to contamination, as pesticide residues bioaccumulate in livestock tissues used as protein sources in pet food (MacLachlan and Bhula, 2009). This dual exposure from both plant- and animal-based ingredients underscores the need for systematic monitoring of pesticide residues across all components of pet food formulations.

Given the extensive use of pesticides in agriculture, their detection in pet food is unsurprising. Previous studies have identified pesticide residues in the serum, hair, and urine of companion animals, indicating exposure through both dietary and environmental sources (Li et al., 2022; Norrgran Engdahl et al., 2017; Ruiz-Suárez et al., 2015; Wise et al., 2022). Moreover, species-specific metabolic differences influence bioaccumulation, with cats exhibiting higher internal burdens of persistent organic pollutants and pesticide residues than dogs, due to their unique hepatic metabolism (Gautam et al., 2020; Shrestha et al., 2011; Takashima-Uebelhoer et al., 2012).

Chronic pesticide exposure is linked to significant health risks in companion animals, including cancer and endocrine disorders. Epidemiological studies associate pesticide exposure with a higher incidence of malignant lymphoma in dogs (Takashima-Uebelhoer et al., 2012), and transitional cell carcinoma of the bladder, particularly in genetically predisposed animals (Gautam et al., 2020; Luethcke et al., 2019). Furthermore, exposure to pesticide residues has been implicated in the development of mammary tumors in dogs (Wise et al., 2022). In cats, chronic exposure to environmental contaminants, including pesticides, has been strongly associated with hyperthyroidism (Peterson and Ward, 2007). These findings emphasize the need for continuous monitoring and regulatory oversight to mitigate health risks.

Despite the risks posed by pesticide residues in pet food, regulatory frameworks specifically addressing these contaminants in companion animal diets remain insufficient. The European Union's Regulation (EC) No 396/2005 sets maximum residue levels (MRLs) for food and feed of plant and animal origin, primarily targeting livestock production (EC, 2021). Yet, pet food formulations differ significantly from livestock feed in terms of composition, processing, and consumption patterns, making the direct application of these MRLs an inadequate regulatory approach. While some studies have monitored pesticide residues in livestock feed (Kumar et al., 2020; Na et al., 2022), research on pet food contamination remains scarce (Giugliano et al., 2024; Zhao et al., 2018), leaving a critical gap in understanding chronic dietary exposure in companion animals.

Furthermore, current analytical methods for pesticide detection in animal feed are often inadequate for pet food, primarily due to its complex matrix, which includes high-fat and protein-rich plant and animal-derived ingredients. Standard methods designed for livestock feed lack the sensitivity to detect low-level pesticide contamination in pet food, highlighting the need for tailored methodologies that minimize matrix interferences (Eyring et al., 2021; Mol et al., 2008; Musarurwa et al., 2019; van der Lee et al., 2008; Walorczyk and Drozdzyński, 2012). Recent advances in multiresidue analysis, particularly those incorporating QuEChERS-based extraction combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS), have demonstrated improved sensitivity and accuracy in complex food matrices. These approaches allow for the simultaneous detection of a broad spectrum of pesticide residues regulated under European Union legislation, facilitating a more comprehensive assessment of contamination in pet food. The application of such optimized methodologies is essential for generating reliable data on pesticide levels and for better understanding potential risks associated with chronic exposure in companion animals.

This study seeks to bridge this knowledge gap by conducting a comprehensive analysis of pesticide residues in commercial dog and cat food. Specifically, its objectives are to: (i) determine the prevalence of pesticide residues in pet food, (ii) compare detected levels with existing MRLs for livestock feed, and (iii) evaluate the potential toxicological risks associated with chronic exposure in companion animals.

2. Material and methods

2.1. Sampling

A total of 40 commercial dry cat food products (0.8–2 kg) and 43 commercial dry dog food products (1.25–4 kg) were collected from retail outlets, specialized pet stores, and supermarkets in Gran Canaria (Canary Islands, Spain), reflecting the Spain's predominant sales distribution patterns, as reported by distributors. Expiry dates were considered, and samples with less than six months of remaining shelf life at the time of purchase were included.

Both well-established commercial brands and private-label brands (supermarket brands) were included, due to their significant market share and widespread consumer preference. To ensure a balanced representation across cost tiers, wholesaler and distributor price data were obtained via telephone inquiries. Since the unit price per kilogram varies with packaging size, prices from different packaging formats of each brand were averaged to obtain a representative €/kg value. The median €/kg value was used to classify pet food products into two quality categories: high and low quality. Products priced above the median were categorized as high-quality, while those priced below were classified as low-quality. This price-based stratification thus reflects not an intrinsic measure of quality, but rather the positioning strategy adopted by manufacturers and perceived by consumers.

Additionally, at least 40 % of the selected samples contained vegetables among their listed ingredients to ensure adequate representation of plant-based formulations. Bulk pet food products were excluded from the study. While all samples were acquired in Gran Canaria, none of the selected brands were locally manufactured; instead, only nationally and internationally distributed brands were considered. More than 85 % of the selected brands are available throughout the European Union. Until further processing, all samples were stored in their original commercial packaging in a dry, dark environment at room temperature.

2.2. Chemicals and reagents

Analytical-grade acetonitrile (ACN), methanol (MeOH), and formic acid (FA, HCOOH) were purchased from Honeywell (Morristown, NJ, USA). Ultrapure water was obtained from a Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA).

QuEChERS extraction salts ($MgSO_4$, NaCl, CH_3COONa) following the AOAC protocol were acquired from Agilent Technologies (Palo Alto, CA, USA). Glass fiber prefilters (1 μm) and polyester syringe filters (0.20 μm) were purchased from Macherey-Nagel (Düren, Germany).

Certified pesticide standard solutions containing all the pesticides analyzed (211) were procured from CPA Chem (Stara Zagora, Bulgaria) in ten mixed solutions at 100 μg/mL in ACN, ensuring compliance with

the European Union Multiannual Monitoring Plan. Additional individual pesticide standards (purity: 97.1 %–99.9 %) and isotopically labeled internal standards (purity: 99.3 %–99.9 %) were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma-Aldrich (Saint Louis, USA).

Stock solutions of individual pesticides and internal standards were prepared at 1000 μg/mL in ACN and stored in the dark at −20 °C. Working solutions (1 μg/mL) were freshly prepared by appropriate dilutions in ACN. All solutions were periodically checked for stability before use.

2.3. Sample preparation

We have optimized and validated a multiresidue analytical method for the quantification of 211 pesticide residues in commercial dry pet food. The method combines a QuEChERS-based extraction with a refined freeze-out clean-up step, followed by quantitative analysis using liquid chromatography–tandem mass spectrometry (LC-MS/MS) and gas chromatography–tandem mass spectrometry (GC-MS/MS). This approach enhances sensitivity and minimizes matrix interferences, allowing for the reliable detection of a broad spectrum of pesticide residues in pet food.

A 10 ± 0.05 g portion of pet food sample was placed into a 50 mL centrifuge tube, followed by the addition of 10 mL of acetonitrile (ACN) containing 2.5 % formic acid (FA). The mixture was vigorously shaken for 1 min to ensure proper homogenization. Subsequently, 6 g of magnesium sulfate ($MgSO_4$) and 1.5 g of sodium acetate (CH_3COONa) were added, and the tube was shaken again for 1 min, followed by 15 min of sonication in an ultrasonic bath (Selecta, Barcelona, Spain). To enhance extraction efficiency, the samples were then subjected to 25 min of agitation in a rotary shaker (Ovan, Barcelona, Spain).

After this step, the tubes were centrifuged at 4200 rpm (3175.16×*g*) for 10 min using a 5804 R Eppendorf centrifuge (Eppendorf, Hamburg, Germany). The resulting supernatant was passed through 0.20 μm Chromafil® PET filters (Macherey-Nagel, Düren, Germany) and collected in a 5 mL tube. To further purify the extract, a freeze-out clean-up procedure was applied: a 3 mL aliquot was transferred into a 5 mL Eppendorf tube, frozen at −24 °C for 1 h, and then centrifuged at 4200 rpm for 5 min. This process was repeated twice, ensuring the removal of interfering matrix components. Finally, the purified supernatant was transferred into an amber chromatography vial and analyzed using liquid chromatography–tandem mass spectrometry (LC-MS/MS) and gas chromatography–tandem mass spectrometry (GC-MS/MS).

For recovery experiments and quality control procedures, samples were spiked with the appropriate volume of standard mix solutions and 50 μL of P-IS mix solution. Both blank and test samples were left to stand for 1 h before extraction to allow for adequate incorporation of the analytes.

2.4. Quantitative analysis of pesticide residues

Pesticide residues were quantified using both liquid chromatography–tandem mass spectrometry (LC-MS/MS) and gas chromatography–tandem mass spectrometry (GC-MS/MS). LC-MS/MS analyses were performed on a 1290 Infinity II LC System coupled to a Triple Quad 6460 mass spectrometer (Agilent Technologies). Chromatographic separation was carried out using a Poroshell 120 EC-C18 column (2.1 × 100 mm, 2.7 μm; Agilent Technologies) maintained at 50 °C, with a guard prefilter and pre-column. The mobile phase consisted of 2 mM ammonium acetate with 0.1 % formic acid in water (A) and 2 mM ammonium acetate in methanol (B), applied in a binary gradient. The flow rate was set at 0.4 mL/min with a 5 μL injection volume, and the total runtime was 18 min. Detection was conducted using an Agilent Jet Stream Electrospray Ionization Source (AJS-ESI) operating in both positive and negative modes under dynamic multiple reaction monitoring (dMRM). Nitrogen was supplied by an NGMs-1 generator (Atlas Copco, Stockholm, Sweden) as the drying and desolvation gas at 190 °C and 11 L/min, while nitrogen (99.9999 % purity, Linde, Dublin, Ireland) served as the collision gas. The sheath gas was maintained at 330 °C with a flow rate of 12 L/min.

GC-MS/MS analyses were conducted using a GC System 7890B equipped with a 7693 Autosampler and a Triple Quad 7010 mass spectrometer (Agilent Technologies). Chromatographic separation was achieved with two Agilent J&W HP-5MS columns (15 m, 0.25 mm i.d., 0.25 μm film thickness, crosslinked 5 % phenyl-methyl-polysiloxane) connected via a Purged Ultimate Union (PUU; Agilent Technologies) to facilitate back-flushing (−5.8 mL/min, 315 °C for 5 min). Helium (99.999 % purity) was used as the carrier gas, with retention time lock set to chlorpyrifos-methyl (tR = 9.143 min). The column temperature program included an initial hold at 80 °C for 1.8 min, followed by a ramp to 170 °C at 40 °C/min, then to 310 °C at 10 °C/min, with a final hold of 3 min, resulting in a total runtime of 21 min 15 s. Injections were performed in splitless mode (1 μL) using a 4 mm Ultra Inert liner with glass wool at 250 °C. Mass spectrometry was conducted in electron impact (EI) ionization mode under multiple reaction monitoring (MRM), with 24 time segments. The EI source and transfer line were both set at 280 °C, and nitrogen (99.9999 % purity, Linde) was used as the collision gas at a flow rate of 1.5 mL/min with a solvent delay of 3.7 min.

Data analysis was carried out using MassHunter Quantitative Analysis (QQQ) vB.07.01 and MassHunter Qualitative Analysis vB.07.00 (Agilent Technologies) for both LC-MS/MS and GC-MS/MS. Quantification was performed using matrix-matched calibration curves prepared in triplicate, covering a range of 0.002–80 μg/kg, and analyzed on each instrument. Confirmation of analytes was based on the acquisition of two MS/MS transitions, ensuring an ion ratio tolerance within ±30 % between the quantification and confirmation transitions. A maximum retention time deviation of ±0.1 min between the analyte in the sample and the reference standard was considered acceptable. For chiral analytes, results were reported as the sum of all enantiomers unless the residue definition specified otherwise, in which case individual enantiomers were quantified separately.

Quality control measures included the use of procedural internal standards to monitor extraction efficiency and instrumental performance, along with the periodic injection of a quality control of blank matrix spiked with 5 μg/kg with the working mix solutions and extracted using the same procedure as in the samples in every twenty samples.

Detailed chromatographic and mass spectrometric conditions (including retention times, polarity, fragment ions, and collision energies) for all 211 analytes are provided in Supplementary Table 1. Validation parameters for each compound, including limits of detection (LOD), quantification (LOQ), linearity, recovery rates, and precision (RSD%) at multiple spiking levels, are presented in Supplementary Table 2.

2.5. Estimation of dietary exposure and cumulative risk assessment

To estimate the dietary exposure to pesticide residues in companion animals, the estimated daily intake (EDI) was calculated for each pesticide detected in dry pet food samples. The EDI was determined by multiplying the concentration of each pesticide residue in a given sample by the recommended daily consumption (g/kg body weight/day) provided by the manufacturer for that specific product. This approach allowed us to estimate exposure levels relative to body weight basis for both dogs and cats, accounting for species-specific dietary variations.

For each pesticide, exposure levels were expressed as the mean ± standard deviation (SD), median, and range, calculated separately for dogs and cats to reflect differences in dietary consumption patterns and body weight adjustments. To assess potential health risks from chronic exposure, the hazard index (HI) was calculated for each pesticide by dividing the EDI by the tolerable daily intake (TDI) from the European Pesticide Database (EC, 2025). Since no companion animal-specific TDI

values are currently available, human TDIs were used as reference points, in line with previous toxicological risk assessments. Although this approach does not account for potential interspecies differences in metabolism and sensitivity, it provides a conservative and standardized framework for estimating relative risk. An HI value exceeding 1 indicates that exposure surpasses the safe intake level, suggesting a potential health risk that warrants further evaluation.

Beyond individual pesticide assessment, cumulative exposure was evaluated by summing HI values within pesticide classes, including herbicides, fungicides, insecticides, acaricides, and post-harvest preservatives. This classification accounts for differences in modes of action and potential toxicological interactions within each category. Additionally, a total cumulative HI was calculated to provide an overview estimate of pesticide exposure in pet food.

While this cumulative approach provides valuable insights into the potential effects of pesticide mixtures, it relies on several key assumptions. Primarily, it assumes that the toxicological effects of pesticide residues are purely additive, without accounting for potential synergistic interactions that could amplify adverse health outcomes or antagonistic effects that might reduce toxicity. Furthermore, even within the same functional category, pesticides vary significantly in their toxicity, metabolic pathways, and mechanisms of action, making direct comparisons complex. However, although regulatory guidelines addressing for mixture effects are still under evolving, categorizing pesticides by functional group provides a more structured framework for assessing cumulative exposure risks.

2.6. Statistical analysis

All statistical analyses were conducted using GraphPad Prism v10.0 (GraphPad Software, CA, USA). The Kolmogorov-Smirnov test was used to assess variable distribution. Since pesticide residue concentrations did not follow a normal distribution, data are reported as the mean ± standard deviation (SD), median and range, to summarize central tendency and variability.

Differences in pesticide concentrations between categorical groups (e.g., pet species, food quality categories) were analyzed using the non-parametric Mann-Whitney *U* test. A two-tailed p-value <0.05 was considered statistically significant. Additionally, Spearman's rank correlation was applied to explore potential associations between pesticide concentrations and product characteristics (e.g., price, the presence of plant-based ingredients).

3. Results

3.1. Occurrence of pesticide residues in dry pet food

A total of 83 dry pet food samples (40 for cats and 43 for dogs) were analyzed for the presence of 211 pesticide residues. Of these, 51 different pesticide residues were detected in at least one sample, representing 24.2 % of the targeted compounds. The detected pesticides spanned multiple chemical classes, including fungicides (24), insecticides (19), acaricides (4), herbicides (3), and one post-harvest preservative (Fig. 1A). Fungicides were the most frequently detected pesticide class, comprising 47 % of all detected residues, followed by insecticides (37 %).

A key finding of this study was the high prevalence of unapproved pesticides in the analyzed samples. Among the 51 detected residues, 19 (37.3 %) are not currently authorized for use in the European Union (Fig. 1B). These include long-banned substances such as atrazine (herbicide), chlorpyrifos (insecticide), and carbendazim (fungicide), which were prohibited due to their environmental persistence and potential toxicity to non-target species.

Among the detected pesticides, 19 exceeded the general maximum residue limit (MRL) of 0.01 mg/kg (10 μg/kg) set for feed materials of plant and animal origin under Commission Regulation (EC) No 396/2005 (EC, 2021). The most frequently detected compounds above this threshold included pirimiphos-methyl, found in 21 samples (13 dog foods and 8 cat foods), with maximum concentrations reaching 184.3 μg/kg, and chlorpyrifos-methyl, detected in 29 samples (16 dog foods and 13 cat foods), with levels up to 15.1 μg/kg. Notably, pirimiphos-methyl—a widely used organophosphate insecticide—had the highest concentration among all residues.

Several banned pesticides were also detected above or close to the 10 μg/kg threshold, raising concerns about regulatory compliance. Chlorpyrifos-methyl, prohibited in the EU since 2020 due to its neurotoxic effects, was present in 29 samples at concerning levels. Carbendazim, a fungicide banned due to its endocrine-disrupting properties, was found at 6.3 μg/kg in a cat food sample, while chlorpropham, an herbicide banned since 2020, reached 14.5 μg/kg in a dog food sample.

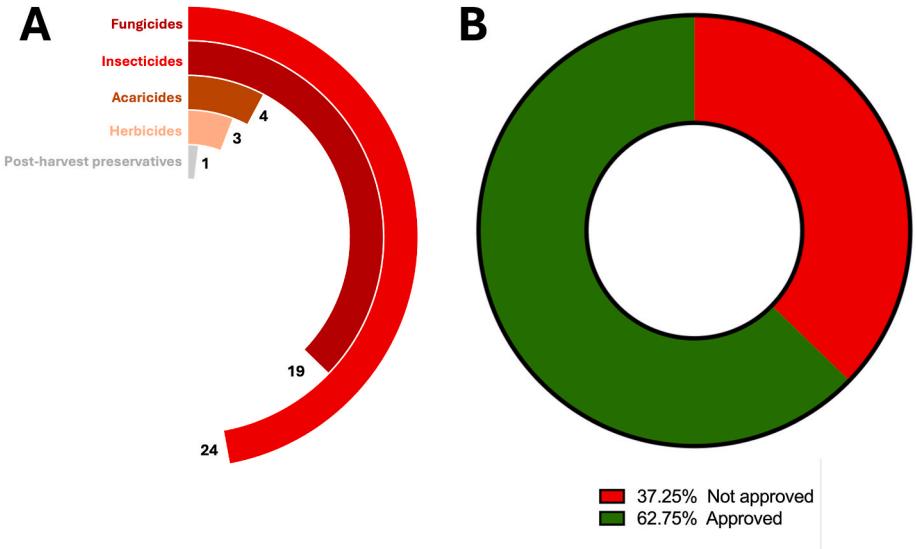

Fig. 1. Distribution and regulatory status of pesticide residues detected in dry pet food samples. (A) Classification of the 51 detected pesticides by chemical class: fungicides (n = 24), insecticides (n = 19), acaricides (n = 4), herbicides (n = 3), and one post-harvest preservatives (n = 1). (B) Proportion of detected pesticides approved (green, 62.75 %) or unapproved (red, 37.25 %) for use in the European Union, according to the European Pesticide Database (EC, 2025). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The descriptive statistics for each detected pesticide, including mean, median, and concentration range, are summarized in Table 1. The comparison of pesticide residue prevalence between dog and cat food samples revealed no statistically significant differences in total residue levels (Fig. 2, right panel). However, the number of pesticide residues per sample was significantly higher in dog food than in cat food ($p = 0.0145$, Fig. 2, left panel), suggesting a broader range of contamination in canine diets. On average, dog food samples contained 5.7 ± 4.8 residues per sample, with a median of 4 residues, whereas cat food samples contained 4.3 ± 5.1 residues per sample, with a median of 3 residues. Notably, some dog food brands exhibited particularly high contamination levels, with up to 20 pesticide residues detected in a single dog food sample and 21 in a single cat food sample, respectively.

A statistically significant difference in total pesticide concentrations was observed between dry pet food formulations with and without plant-derived ingredients ($p = 0.04106$, Mann-Whitney *U* test; Fig. 3). Pet foods containing vegetables, fruits, or cereals had higher total pesticide concentrations than those formulated exclusively with animal-based ingredients. This finding aligns with the widespread use of pesticides in crop production and post-harvest treatments, suggesting that plant-based components in pet food are a primary source of pesticide residues.

In contrast, no significant differences were found in pesticide residues based on brand type (supermarket store-brand vs. commercial brand) or price category (high vs. low). Despite the common perception that premium-priced pet foods may adhere to stricter quality standards, our results indicate that pesticide contamination is independent of product price. Similarly, the lack of differences between supermarket and commercial brands suggests that regulatory compliance and ingredient sourcing practices do not vary substantially between these categories.

3.2. Estimated daily intake (EDI) and hazard index (HI)

The estimated daily intake (EDI) of pesticide residues from dry pet food consumption varied considerably among compounds and between

Table 1
Descriptive statistics of pesticide residues in commercial dry dog and Cat food (μg/kg).

Pesticide	Dog Food						Cat Food						*P*
	N	Mean	SD	Median	Min	Max	N	Mean	SD	Median	Min	Max	
Fenazaquin							3.0	18.1	9.6	17.9	8.5	27.7	
Hexythiazox	1.0	0.8	0.0	0.8	0.8	0.8	1.0	1.2	0.0	1.2	1.2	1.2	
N.N-dimethylformamidine (DMF. metabolite of amitraz)							1.0	3.1	0.0	3.1	3.1	3.1	
Tebufenpyrad							1.0	0.3	0.0	0.3	0.3	0.3	
2-Phenylphenol	9.0	6.0	6.1	2.9	1.6	16.6	7.0	2.8	1.6	2.4	1.6	6.1	
Azoxystrobin	15.0	0.4	0.6	0.2	0.0	2.4	6.0	0.2	0.1	0.2	0.1	0.4	
Boscalid	6.0	4.2	3.2	4.2	0.5	7.8	5.0	3.3	3.6	1.4	0.3	8.9	
Carbendazim	5.0	1.0	0.9	0.5	0.3	2.5	1.0	6.3	0.0	6.3	6.3	6.3	
Cyflufenamid							1.0	0.2	0.0	0.2	0.2	0.2	
Cyprodinil	6.0	0.6	0.2	0.6	0.4	0.9	2.0	1.6	0.8	1.6 *	1.1	2.2	**0.0414**
Difenoconazole	13.0	1.5	1.5	1.1	0.7	6.6	6.0	1.6	1.1	1.0	0.8	3.5	
Epoxiconazole	9.0	0.3	0.2	0.3	0.2	0.7	4.0	0.4	0.2	0.4	0.2	0.5	
Fenpropidin	3.0	0.2	0.1	0.2	0.2	0.3	1.0	0.2	0.0	0.2	0.2	0.2	
Fenpropimorph	1.0	0.3	0.0	0.3	0.3	0.3	1.0	1.6	0.0	1.6	1.6	1.6	
Fludioxonil	6.0	5.6	4.1	7.3	0.2	9.8	4.0	5.4	4.8	5.3	0.8	10.1	
Fluopyram	7.0	1.7	1.5	1.0	0.4	4.5	3.0	1.0	1.2	0.5	0.2	2.3	
Flutolanil	3.0	0.3	0.1	0.3	0.3	0.4	3.0	0.3	0.1	0.2	0.2	0.4	
Imazalil (Enilconazole)	6.0	0.8	0.2	0.8	0.6	1.2	5.0	4.3	7.7	1.1	0.3	18.1	
Isoprothiolane	5.0	0.5	0.2	0.6	0.2	0.7	2.0	1.0	0.3	1.0	0.8	1.2	
Mandipropamid							1.0	0.3	0.0	0.3	0.3	0.3	
Metalaxyl	1.0	0.1	0.0	0.1	0.1	0.1							
Metrafenone	11.0	0.4	0.1	0.4	0.3	0.6	10.0	0.6	0.3	0.5	0.3	1.2	
Pyraclostrobin	9.0	2.0	2.2	0.8	0.2	6.1	5.0	4.3	5.3	1.6	0.2	12.1	
Pyrimethanil	8.0	2.0	2.3	1.0	0.2	6.8	7.0	3.1	3.9	1.5	0.1	10.3	
Spiroxamine	10.0	0.2	0.2	0.1	0.1	0.7	5.0	0.1	0.0	0.1	0.1	0.1	
Thiabendazole	2.0	0.9	0.3	0.9	0.7	1.1	2.0	0.8	0.1	0.8	0.7	0.9	
Thiophanate-methyl	1.0	0.4	0.0	0.4	0.4	0.4	1.0	0.4	0.0	0.4	0.4	0.4	
Trifloxystrobin	9.0	0.3	0.2	0.3	0.2	0.8	8.0	0.3	0.2	0.2	0.2	0.6	
Atrazine	2.0	0.2	0.0	0.2	0.2	0.3							
Chlorpropham	6.0	4.9	5.4	3.0 *	0.7	14.5	4.0	0.7	0.5	0.5	0.4	1.5	**0.0380**
Diflufenican	2.0	0.2	0.0	0.2	0.2	0.2							
Acetamiprid							1.0	0.9	0.0	0.9	0.9	0.9	
Buprofezin	2.0	1.4	0.2	1.4	1.3	1.6							
Chlorantraniliprole	1.0	1.0	0.0	1.0	1.0	1.0	2.0	1.7	0.1	1.7	1.7	1.8	
Chlorpyrifos	24.0	1.2	0.9	1.1	0.2	3.6	16.0	0.8	0.6	0.5	0.2	2.3	
Chlorpyrifos-methyl	16.0	1.6	1.4	0.9	0.3	4.7	13.0	4.7	4.9	3.1	0.4	15.1	
Fenoxycarb	3.0	0.3	0.0	0.3	0.3	0.4							
Lufenuron	1.0	1.2	0.0	1.2	1.2	1.2	2.0	0.7	0.0	0.7	0.7	0.7	
Malathion	3.0	0.8	0.5	0.5	0.4	1.4	1.0	0.3	0.0	0.3	0.3	0.3	
Methoxyfenozide	3.0	0.1	0.0	0.1	0.1	0.1	2.0	0.1	0.1	0.1	0.1	0.2	
Phosalone	1.0	0.8	0.0	0.8	0.8	0.8							
Phosmet	2.0	1.0	0.0	1.0	1.0	1.1	1.0	1.6	0.0	1.6	1.6	1.6	
Pirimicarb	5.0	0.1	0.1	0.1	0.1	0.2	4.0	0.3	0.2	0.2	0.1	0.5	
Pirimiphos-methyl	13.0	37.3	48.6	14.4	3.4	159.0	8.0	57.5	70.7	19.8	4.3	184.3	
Propoxur	1.0	0.4	0.0	0.4	0.4	0.4	1.0	0.3	0.0	0.3	0.3	0.3	
Pyriproxyfen							2.0	0.7	0.4	0.7	0.4	1.0	
Tebuconazole	20.0	1.2	1.1	0.7	0.2	3.7	11.0	1.4	2.1	0.5	0.2	7.4	
Tebufenozide	3.0	0.8	0.2	0.8	0.6	0.9							
Thiacloprid	3.0	0.3	0.1	0.3	0.3	0.4							
Triazophos (hostathion)	1.0	0.2	0.0	0.2	0.2	0.2	1.0	0.2	0.0	0.2	0.2	0.2	

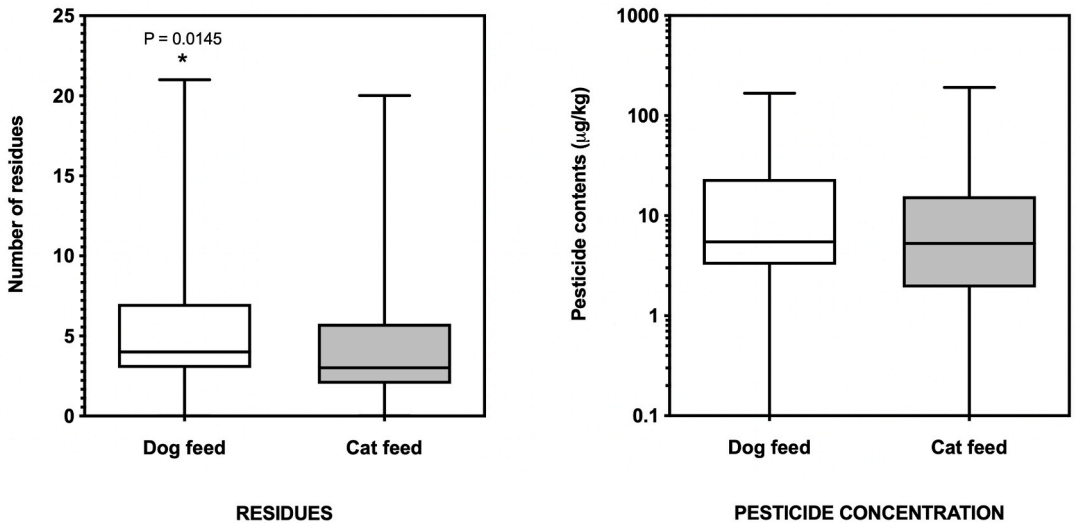

Fig. 2. Comparison of pesticide residues and total concentrations in dog and cat food samples. Box plots represent (left) the number of detected pesticide residues per sample and (right) total pesticide concentrations (µg/kg). The central line in each box represents the median, the box spans the interquartile range (IQR), and the whiskers extend to the minimum and maximum values within 1.5 times the IQR.

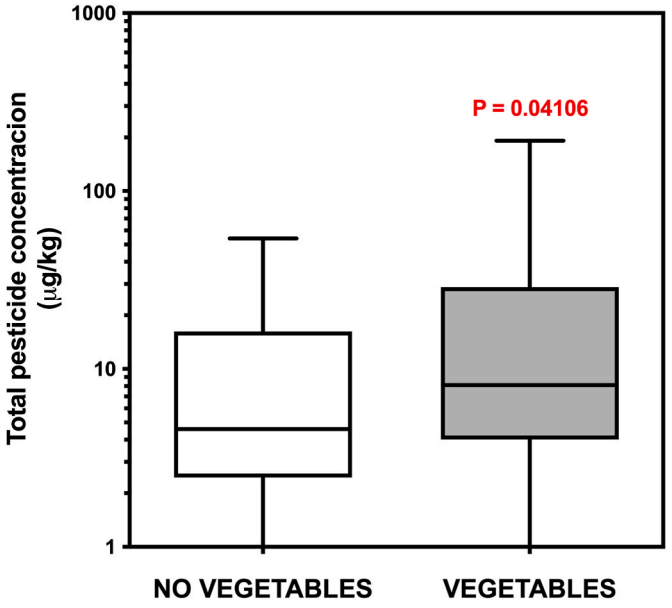

Fig. 3. Total pesticide concentrations (µg/kg) in dry pet food samples based on the presence of plant-derived ingredients. The central line in each box represents the median, the box spans the interquartile range (IQR), and the whiskers extend to the minimum and maximum values within 1.5 times the IQR.

species (Table 2). The mean total EDI for dogs was 1224.5 ng/kg bw/day, whereas for cats, it was slightly higher at 1309.2 ng/kg bw/day. However, significant differences were observed for specific pesticide groups. Insecticides accounted for the highest proportion of daily intake in both species, averaging 657.3 ng/kg bw/day in dogs and 726.5 ng/kg bw/day in cats. Fungicides were the second most relevant category, contributing with 412.9 ng/kg bw/day in dogs and 366.1 ng/kg bw/day in cats. The intake of acaricides was significantly higher in cats than in dogs ($p = 0.0388$), whereas herbicide intake remained relatively low in both species (Fig. 4).

Among the detected residues, pirimiphos-methyl, an organophosphate widely used as a grain protectant, exhibited the highest EDI values in both species, with a mean intake of 920.8 ng/kg bw/day in dogs and 1047.0 ng/kg bw/day in cats. Other compounds with notably high EDI values included chlorpyrifos-methyl, fludioxonil, and 2-phenylphenol. In contrast, several pesticides exhibited low but detectable intakes, such as thiophanate-methyl, metalaxyl, and mandipropamid, with mean EDIs below 10 ng/kg bw/day.

The Hazard Index (HI) was calculated to assess the potential risk posed by cumulative pesticide exposure (Fig. 5). The overall HI values for both species remained well below the threshold of 1, with the worst-case scenario being 0,32 for total exposure. This indicates that, based on current reference doses, estimated exposure levels do not pose an immediate toxicological risk. However, among individual pesticide categories, insecticides contributed the most to the cumulative HI, followed by fungicides and acaricides. Notably, the HI values were comparable between dog and cat food, suggesting that both species experience a similar level of risk from chronic dietary pesticide exposure.

4. Discussion

The detection of pesticide residues in dry pet food raises concerns regarding chronic dietary exposure in companion animals. Our findings indicate that 24.2 % of the targeted 211 pesticides were detected in at least one sample, with a substantial proportion belonging to the fungicide (47 %) and insecticide (37 %) categories. The predominance of fungicides is not unexpected, as these compounds are widely used in agriculture to prevent mold contamination and extend the shelf life of grains and vegetables, key ingredients in many pet food formulations. These residues have also been previously detected in other animal feed samples (Giugliano et al., 2024; Penagos-Tabares et al., 2023). However, the high incidence of banned substances (37.3 % of detected pesticides) highlights the need for stricter regulatory enforcement and systematic monitoring of pesticide residues in pet food products.

Despite the well-documented occurrence of pesticide residues in agricultural commodities and livestock feed (Bedi et al., 2018; Giugliano et al., 2024; Li and Fantke, 2023; Penagos-Tabares et al., 2023; Zhang et al., 2015), studies evaluating their presence in pet food remain scarce (Ruiz-Suárez et al., 2015; Zhao et al., 2018). To our knowledge, this is one of the first comprehensive investigations assessing both pesticide

Table 2
Estimated pesticide exposure in dogs and cats (ng/kg bw/day) from commercial dry pet food consumption.

Pesticide	DOGS					CATS					
	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max	*P*
Fenazaquin						169.8	149.9	155.1	27.7	326.5	
Hexythiazox	18.5	0.0	18.5	18.5	18.5	21.1	0.0	21.1	21.1	21.1	
N.N-dimethylformamidine (DMF. metabolite of amitraz)						56.1	0.0	56.1	56.1	56.1	
Tebufenpyrad						5.6	0.0	5.6	5.6	5.6	
2-Phenylphenol	147.3	150.6	**71.9** *	39.5	409.0	50.8	28.6	43.7	28.9	111.7	**0.0449**
Azoxystrobin	9.5	14.2	5.7	1.0	58.5	3.9	2.3	3.2	1.8	7.5	
Boscalid	103.9	79.1	104.9	11.4	193.4	59.9	65.2	24.9	6.0	162.7	
Carbendazim	25.8	22.8	12.8	6.9	61.3	115.0	0.0	115.0	115.0	115.0	
Cyflufenamid						4.2	0.0	4.2	4.2	4.2	
Cyprodinil	14.4	4.7	13.6	9.4	21.0	29.8	14.3	29.8	19.7	39.9	
Difenoconazole	38.1	38.2	26.2	18.0	162.3	29.3	19.9	19.0	14.7	63.2	
Epoxiconazole	8.2	4.5	6.7	4.2	18.3	6.5	2.8	7.0	2.9	8.9	
Fenpropidin	5.3	1.9	4.2	4.2	7.4	3.3	0.0	3.3	3.3	3.3	
Fenpropimorph	8.2	0.0	8.2	8.2	8.2	28.8	0.0	28.8	28.8	28.8	
Fludioxonil	139.5	102.2	179.9	4.4	242.1	97.5	87.8	96.6	13.7	183.1	
Fluopyram	41.8	37.4	24.7	10.6	110.4	18.1	21.2	8.6	3.3	42.4	
Flutolanil	8.2	1.7	7.4	6.9	10.1	4.8	2.2	4.2	2.9	7.3	
Imazalil (Enilconazole)	20.4	5.5	18.6	14.8	29.9	78.4	140.1	19.3	4.7	328.7	
Isoprothiolane	13.0	5.7	15.6	4.0	18.3	18.4	5.4	18.4	14.6	22.2	
Mandipropamid						4.7	0.0	4.7	4.7	4.7	
Metalaxyl	2.0	0.0	2.0	2.0	2.0						
Metrafenone	10.4	2.6	9.9	6.4	13.6	10.6	5.1	9.4	5.8	20.9	
Pyraclostrobin	49.1	54.2	20.0	4.2	149.4	79.1	96.3	29.8	3.5	220.0	
Pyrimethanil	48.8	56.0	25.8	4.2	167.2	57.0	71.1	27.3	1.6	186.6	
Spiroxamine	4.0	4.3	**2.7** *	2.0	16.1	1.6	0.2	1.6	1.5	1.8	**0.0003**
Thiabendazole	21.9	6.5	21.9	17.3	26.4	14.2	1.8	14.2	12.9	15.5	
Thiophanate-methyl	8.6	0.0	8.6	8.6	8.6	7.5	0.0	7.5	7.5	7.5	
Trifloxystrobin	8.5	5.3	**6.4***	4.0	20.0	5.3	3.1	3.9	2.9	10.9	**0.0345**
Atrazine	6.1	0.2	6.1	5.9	6.2						
Chlorpropham	120.7	132.3	**73.7** *	16.3	358.2	13.0	9.2	9.0	7.3	26.8	**0.0381**
Diflufenican	5.7	0.0	5.7	5.7	5.7						
Acetamiprid						16.6	0.0	16.6	16.6	16.6	
Buprofezin	34.8	5.6	34.8	30.9	38.8						
Chlorantraniliprole	25.7	0.0	25.7	25.7	25.7	30.9	1.3	30.9	30.0	31.9	
Chlorpyrifos	29.5	21.2	**26.9** *	4.2	87.9	14.4	11.3	9.6	3.3	41.3	**0.0166**
Chlorpyrifos-methyl	39.3	34.1	23.1	7.9	116.3	86.0	88.6	56.6	6.6	274.6	
Fenoxycarb	7.6	1.2	7.2	6.7	8.9						
Lufenuron	30.1	0.0	30.1	30.1	30.1	12.3	0.6	12.3	11.8	12.7	
Malathion	18.7	13.1	11.6	10.6	33.8	5.6	0.0	5.6	5.6	5.6	
Methoxyfenozide	1.8	0.8	1.5	1.2	2.7	2.0	1.0	2.0	1.3	2.7	
Phosalone	19.8	0.0	19.8	19.8	19.8						
Phosmet	25.8	0.5	25.8	25.4	26.2	28.9	0.0	28.9	28.9	28.9	
Pirimicarb	3.6	1.9	2.5	2.0	5.9	4.8	3.5	4.5	1.8	8.4	
Pirimiphos-methyl	920.8	1200.9	354.7	83.5	3926.8	1047.0	1285.9	361.2	78.1	3353.9	
Propoxur	9.4	0.0	9.4	9.4	9.4	6.0	0.0	6.0	6.0	6.0	
Pyriproxyfen						12.7	8.0	12.7	7.1	18.4	
Tebuconazole	29.7	27.2	17.8	4.0	90.6	26.1	38.7	8.7	2.9	134.5	
Tebufenozide	18.9	4.2	20.5	14.1	22.0						
Thiacloprid	8.2	1.9	7.7	6.7	10.4						
Triazophos (hostathion)	4.4	0.0	4.4	4.4	4.4	3.5	0.0	3.5	3.5	3.5	

prevalence and potential dietary exposure in companion animals. Previous research has primarily focused on the presence of contaminants in livestock feed, with limited attention given to pet food safety. This knowledge gap is particularly relevant due to the distinct dietary patterns and metabolic peculiarities of dogs and cats, which differ significantly from those of farm animals. Our findings underscore an urgent need for further studies on pesticide exposure in pets, considering their prolonged and often exclusive consumption of commercial diets (Laflamme et al., 2008).

One of the most concerning findings was the detection of multiple pesticides banned in the EU for years due to their toxicity and environmental persistence (EC, 2025). Compounds such as chlorpyrifos, carbendazim, and atrazine have been linked to neurotoxicity, endocrine disruption, and long-term ecological damage (Singh et al., 2016; Stradtman and Freeman, 2021; Ubaid ur Rahman et al., 2021). The continued presence of these substances in commercial pet food suggests potential contamination of raw materials, insufficient regulatory oversight, or unauthorized use in certain agricultural practices. As pets consume the same diet daily over extended periods (Laflamme et al., 2008), even low-level chronic exposure to these pesticides may pose health risks, particularly in species with limited detoxification capacities, such as cats (Shrestha et al., 2011).

The comparison between dog and cat food revealed a significantly higher number of pesticide residues in dog food samples, with certain brands exhibiting particularly high contamination levels, containing over 20 different pesticide residues in a single sample. This difference may arise from variations in ingredient sourcing and formulation, as dog food often contains a more diverse range of plant-derived components, such as cereals and vegetable-based proteins, which are more likely to be treated with pesticides (Zhao et al., 2018). In contrast, cat food formulations are predominantly meat-based, potentially reducing the introduction of plant-related pesticide residues (Badri et al., 2021). However, despite this difference in the number of detected residues, the total pesticide concentration in dog and cat food was not significantly different, suggesting that while dog food may contain more individual pesticide compounds, cat food samples can still reach comparable

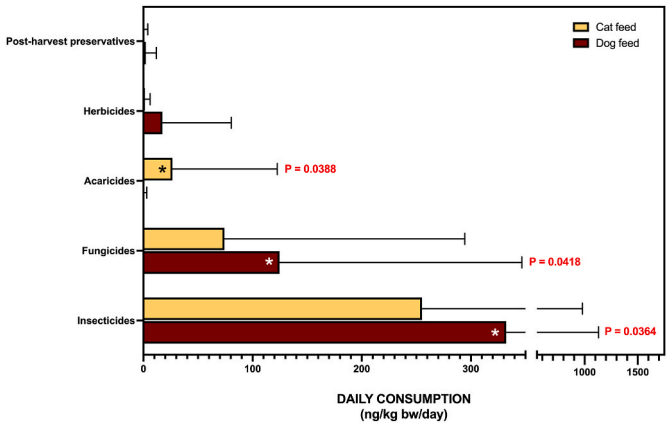

Fig. 4. Estimated daily intake (EDI) of pesticide residues in dogs and cats from dry pet food consumption. the mean daily intake (ng/kg bw/day) is presented for each pesticide category, comparing dog and cat food. Statistically significant differences between species are indicated with * ($p < 0.05$).

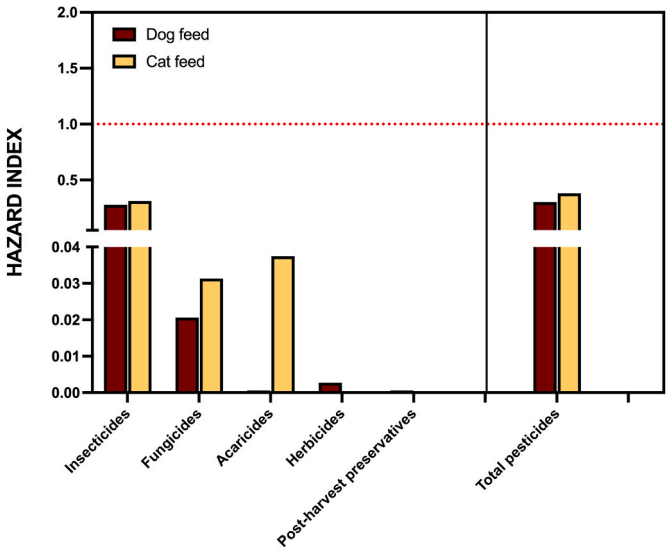

Fig. 5. Hazard Index (HI) for Pesticide Exposure in Dry Pet Food. The HI was calculated for different pesticide categories (insecticides, fungicides, acaricides, herbicides, and post-harvest preservatives) and for total pesticide exposure in dog and cat food. The red dotted line represents the threshold value of 1, above which potential health risks may be considered significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

overall pesticide burdens.

The significant association between higher pesticide concentrations and the presence of plant-based ingredients supports the hypothesis that cereals, fruits, and vegetables in pet food contribute substantially to pesticide exposure. This aligns with previous studies, showing that grains and legumes in pet food formulations often contain pesticide residues due to pre- and post-harvest treatments (Giugliano et al., 2024; Walorczyk and Drozdzyński, 2012). While plant-derived ingredients may provide nutritional benefits (Badri et al., 2021; FEDIAF, 2024; Guo et al., 2024; Tanprasertsuk et al., 2022), their inclusion also represents a potential risk factor for increased exposure to pesticide residues. Despite the consumer perception that premium pet food brands offer better quality control (Vinassa et al., 2020), our findings showed no significant differences in pesticide concentrations between premium-priced and lower-priced products. Likewise, no statistically significant differences were observed between private-label (supermarket) brands and well-known commercial brands, suggesting that price and branding may not be reliable indicators of pesticide contamination levels in pet food, as previously reported for other pollutants (Macías-Montes et al., 2021, 2020).

The EDI analysis revealed that the total pesticide intake was slightly higher in cats than in dogs, with significant differences found in certain pesticide categories. Insecticides accounted for the highest proportion of daily intake in both species, followed by fungicides and acaricides. Notably, acaricide intake was significantly higher in cats, which may reflect differences in ingredient composition between cat and dog food formulations. The detection of pirimiphos-methyl and chlorpyrifos-methyl at concentrations exceeding the general 0.01 mg/kg MRL is particularly noteworthy. Pirimiphos-methyl, an organophosphate insecticide widely used to protect stored grains (Weng et al., 2019), was frequently detected at relatively high concentrations, suggesting persistent contamination of raw materials. Chlorpyrifos-methyl, another organophosphate with well-documented neurotoxic effects (Ubaid ur Rahman et al., 2021), was also present in a considerable number of samples despite its EU ban in 2020 (EC, 2025). This raises the possibility of imported ingredients originating from countries where these substances remain authorized or cross-contamination occurring during processing and storage. Similarly, the presence of carbendazim, chlorpropham, and atrazine—banned due to concerns over endocrine disruption, reproductive toxicity, and groundwater contamination (Fujitani et al., 2000; Singh et al., 2016; Stradtman and Freeman, 2021)—highlights potential gaps in the regulatory control of pet food supply chains.

Although the hazard index (HI) values calculated in this study remained below the critical threshold of 1, indicating that individual pesticide exposure levels do not pose an immediate toxicological risk, chronic exposure effects remain uncertain (Lukowicz et al., 2018; Nougadère et al., 2012). Current regulatory frameworks primarily assess the toxicity of individual pesticide compounds, yet real-world exposure involves complex mixtures that may lead to additive or synergistic effects (Takakura et al., 2013). The presence of multiple residues in a single sample suggests that companion animals may be subjected to combined toxicological burdens that are not yet fully understood (Crépet et al., 2019; Kennedy et al., 2019; Lukowicz et al., 2018; Takakura et al., 2013). This is particularly relevant for cats, which exhibit limited hepatic glucuronidation capacity (Shrestha et al., 2011), making them more susceptible to the bioaccumulation of certain xenobiotics. Furthermore, chronic exposure to low doses of neurotoxic and endocrine-disrupting pesticides has been linked to various health concerns in humans and wildlife (Antonangeli et al., 2023; Birnbaum, 2012; Campos and Freire, 2016), raising concerns about potential long-term effects in pets. Considering these uncertainties, future studies must investigate the cumulative risks of prolonged dietary pesticide exposure in companion animals.

From a regulatory standpoint, our findings highlight the urgent need for stricter monitoring and enforcement of pesticide residue limits in pet food. While the European Union has established MRLs for pesticides in livestock feed (EC, 2021), which are extended to pet food by default, there is currently no dedicated regulatory framework specifically addressing pesticide contamination in these products. Other regions, including the United States, similarly lack clear or enforceable regulations regarding pesticide residues in pet food. The absence of specific guidelines for companion animal diets represents a significant regulatory gap that should be addressed to ensure the long-term safety of pets. Establishing clear MRLs for pet food, along with stricter oversight of imported ingredients—particularly through routine residue screening of plant-based inputs and improved traceability systems— would help mitigate the risk of pesticide exposure in companion animals and align regulatory practices with growing consumer demand for transparency in pet nutrition (Kumcu and Woolverton, 2015; Vinassa et al., 2020).

5. Conclusions

This study provides one of the first comprehensive assessments of pesticide residues in commercial pet food, underscoring the urgent need for enhanced regulatory oversight and quality control to address the presence of both approved and banned substances. Our findings indicate that 24.2 % of the 211 targeted pesticides were detected in at least one sample, with fungicides and insecticides being the predominant categories. The presence of non-approved pesticides, such as chlorpyrifos, carbendazim, and atrazine, raises regulatory concerns regarding raw material contamination materials and potential weaknesses in enforcement mechanisms.

Despite variations in the number of residues detected, the overall pesticide concentration did not significantly differ between dog and cat food. The presence of plant-derived ingredients was significantly associated with higher pesticide concentrations, underscoring the importance of ingredient selection in pet food safety. Notably, the estimated dietary exposure and calculated Hazard Index (HI) suggest that chronic exposure to pesticide residues in pet food remains below established risk thresholds. However, current risk assessments do not fully account for cumulative effects or potential interactions between multiple residues, particularly in species with distinct metabolic pathways, such as cats.

This study also identifies a significant gap in regulatory frameworks, as no specific maximum residue limits (MRLs) exist for pet food in the European Union. While existing MRLs for human food and livestock feed are sometimes used as references, their applicability to pet diets remains uncertain, given the unique dietary habits and metabolic traits of companion animals. Stricter pet food-specific regulations, alongside enhanced monitoring of pesticide residues, are essential to ensure consumer confidence and long-term animal health.

Future research should focus on long-term exposure studies to better understand the cumulative and potential synergistic effects of pesticide residues in pet diets. Additionally, further investigations into the bioaccumulation of specific compounds in companion animals and their potential health implications are warranted. Expanding surveillance efforts to include a broader range of pet food formulations and geographical markets will also be crucial in refining risk assessments and informing regulatory policies.

CRediT authorship contribution statement

Ana Macías-Montes: Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Octavio P. Luzardo:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Annalisa Zaccaroni:** Writing – review & editing, Investigation, Data curation. **Andrea Acosta-Dacal:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT Model 4o to improve the clarity and fluency of the manuscript, as English is not our native language. After using this tool, the authors carefully reviewed and edited the content as needed and take full responsibility for the final version of the publication.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.126399.

Data availability

We have uploaded our dataset to Mendeley Data

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