sustainability

Article

Organochlorine Pesticides in Dairy Cows' Diet and the Carryover into Milk in NW Romania

Mirela Miclean [1], Erika Andrea Levei [2] and Oana Cadar [2,*]

[1] Somes–Tisa Water Basin Administration, 17 Vanatorului Street, 400213 Cluj Napoca, Romania; mirelamiclean@yahoo.com
[2] INCDO–INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath Street, 400293 Cluj Napoca, Romania; erika.levei@icia.ro
* Correspondence: oana.cadar@icia.ro

Abstract: Since modern agriculture relies on high volumes of chemical pesticides, monitoring the exposure to these dangerous substances in feed and the food chain is crucial. This study investigates the transfer of organochlorine pesticide (OCP) from water, soil, and feed to milk in free-range dairy cows using the carryover rates (CORs) and biotransfer factors (BTFs) from feed to milk as tools that can be used for human and animal risk assessments. BTFs can predict the transfer of OCPs into milk due to cows' ingestion of contaminated feed. Samples were collected from ten small-scale dairy cow farms located near Baia Mare city, NW Romania, identified for distributing fresh milk in the local market. After the appropriate extraction was performed, the OCP concentrations were measured by gas chromatography equipped with an electron capture detector and randomly confirmed using mass spectrometry. The most prominent compounds in the soil, feed, and milk samples were hexachlorocyclohexane (HCH) isomers and dichlorodiphenyltrichloroethane (DDT) and its metabolites. The concentrations of OCPs were lower than the maximum admissible values in feed, water, soil, and milk. The CORs of OCPs varied over four orders of magnitudes, from 0.10% (2,4′–dichlorodiphenylchloroethylene, 2,4′–DDE) to 250% (4,4′–dichlorodiphenyltrichloroethane, 4,4′–DDT). Similar to CORs, the values of BTFs varied largely, from 0.0001 to 1.408. Additionally, the values higher than the unit for some BTFs could be due to the accumulation and biomagnification of these organochlorine compounds in the animal body. The cows' dietary exposure was evaluated by the estimated daily intake (EDI), considering the three components of ingestion (feed, water, and soil), and varied in the range of 0.0008–0.3509 µg/day/body weight (bw).

Keywords: organochlorine pesticides; water; soil; feed; cow milk; carryover; dietary exposure

Citation: Miclean, M.; Levei, E.A.; Cadar, O. Organochlorine Pesticides in Dairy Cows' Diet and the Carryover into Milk in NW Romania. *Sustainability* **2024**, *16*, 434. https://doi.org/10.3390/su16010434

Academic Editors: Yi-Shiou Chiou, Chi-Wei Huang and Hung-Yin Lin

Received: 24 November 2023
Revised: 29 December 2023
Accepted: 31 December 2023
Published: 3 January 2024

1. Introduction

The global contamination of the environment with various chemicals has raised concerns in the scientific community [1,2]. Among these contaminants, endocrine disruptors are an important class of synthetic chemicals capable of interfering with the natural hormonal processes of human or animal bodies and can produce toxic effects, even at extremely low doses [3,4]. All these chemicals bioconcentrate and biomagnify through the food chain, producing numerous adverse effects (i.e., carcinogenic, reproductive, neurological, and immunological) for both animals and humans [5].

In recent decades, agricultural chemicals, pesticides, and veterinary drugs have been widely used in food production [4]. Organochlorine pesticides (OCPs) are widespread in the environment and have received considerable attention in recent decades, being proven to be ubiquitous and toxic, and to have long persistence and low biodegradability [6–8]. They act as system toxicants and endocrine disruptors, interfering with the normal function of the endocrine system of both wildlife and humans, thus resulting in cancer and reproduction disorders, and being accountable for a wide range of toxic effects, like immunotoxicity and teratogenicity [6]. OCPs were widely used worldwide, mainly to enhance crop production and agricultural productivity [2]. Although most OCPs were banned or their use was drastically limited in most countries around the world [9–11], they are still produced and used in some developing countries as they are cheap, easy to obtain, and highly effective [2,12]. Due to their high persistence, they persevere in the environment, even after the primary sources are eliminated [13]. After their application, the pesticide residues can accumulate in crops, be transported into other environmental areas, such as agricultural soils, water, air, and food chains, and bioaccumulate in different living organisms. The OCP residues present in animal feeds can threaten animal health and reduce the safety of livestock products for human consumption due to their high *n*-octanol–water partition coefficient (K_{ow}), log $K_{ow} > 3.8$, which implies high bioavailability and lipophilicity levels, and high octanol–air partition coefficients (K_{oa}), $7 < \log K_{oa} < 11$, which indicate high bioaccumulation levels in terrestrial food chains [14,15]. As the contamination of feed represents a real and unavoidable issue, there is an imminent need for rational strategies to control and manage the food production chains by implementing good practices in feed production, ensuring their high quality, transport, proper storage, and avoiding further contamination. The maximum admitted concentrations of toxic contaminants in animal feed are regulated by Directive 2002/32/EC as maximum residue levels, i.e., 10 µg/kg for hexachlorobenzene (HCH), β–HCH, aldrin, dieldrin, and total heptachlor; 20 µg/kg for α–HCH; 50 µg/kg for total DDT; 100 µg/kg for the sum of endosulfans; and 200 µg/kg for lindane [16,17].

Livestock is exposed to OCPs by ingesting contaminated feed, water, or soil [18,19]. Depending on the pollutants' bioavailability, this exposure can lead to the contamination of animal products. Milk and dairy products are one of the main contributors to human exposure to persistent organic pollutants since they are among the essential foods for people of all ages in numerous countries around the globe [2,4,20]. Generally known as environmental and food contaminants, persistent and lipophilic pesticides (i.e., lindane) have high bioaccumulation rates in long-life cycle livestock, such as sheep and cows [11,14]. Thus, identifying and quantifying pesticides in various livestock-derived foods are important activities to assess the health risks [21,22].

Pesticide accumulation in cow milk can arise from the carryover processes via contaminated foodstuffs, feed, water, and soil [2,23]. Atmospheric deposition is considered the main route of accumulation of lipophilic chemicals in grass, as plants cannot uptake these chemicals from the soil with their roots and transfer them to the aerial part in substantial amounts. However, since cows ingest soil by grazing, a highly contaminated soil can also be a possible source of OCPs in cow milk and, thus, a main route for human exposure [24,25]. Since OCPs can potentially compromise the health and productivity of animals, monitoring their concentrations in the environment and milk collected from certain areas is important because it provides valuable information on the spatial extent of pollution.

This study is designed to (i) evaluate the concentrations of OCPs in soil, water, feed, and milk from dairy cow farms situated in the Baia Mare area, Maramures County, NW Romania; (ii) calculate the carryover and biotransfer factors (BTFs) of OCPs from feed to milk; and (iii) assess the animal dietary exposure to 19 OCPs through water, soil, and feed consumption.

2. Materials and Methods

2.1. Study Area and Sampling

The Baia Mare area is surrounded by hills and mountains and is characterized by a mild continental climate. Therefore, dairy cows graze for most of the year. Access to pastures provides dairy cows with fresh grass to ingest, leading to healthy cows and a stable production, and high-quality milk with low production costs. Outdoor grazing can also help to reduce ammonia emissions from livestock [26]. To align with the sustainability requirements, the livestock farmers in the studied area face several challenges, such as reducing the environmental impact and greenhouse gas emissions and increasing animal welfare. Half of the studied farms are family farms with up to 25 cows (Romanian Simmental and Holstein Friesian breeds), with long-lasting traditions in raising dairy cows, high care for animal welfare, good pasture management, high product quality transmitted from generation to generation, and are characterized by the self-production of forage for animal feed. The cow waste is used as a natural fertilizer for crops in their vegetable gardens and orchards. In recent years, several ecological improvements were made to enhance the farms' sustainability: three used solar photovoltaic energy, reducing traditional fossil-based energy consumption. All farms are committed to enhancing their sustainability, organic milk production, and environmental management. However, the long-lasting legacy of using chemical fertilizers for intensive production is still experienced at present.

Soil (n = 10), water (n = 10), feed (n = 10), and milk (n = 10) were sampled in September 2022 directly from ten (F1–F10) small-scale, free-range dairy cow farms (Figure 1), identified for frequently distributing cow milk to the market in Baia Mare city, NW Romania. The water samples were collected in triplicate in pre-cleaned Winchester glass bottles from the main water sources (i.e., wells, springs, or community water supplies) used for feeding the cows. Prior to use, the bottles were rinsed with water samples. The water samples were preserved by adding concentrated sulfuric acid, tightly sealed, and stored at 4 °C until the analysis. In each farm, five individual composite soil and feed samples were obtained by mixing five subsamples collected from five different points on the pasture. The soil samples were randomly collected from the upper soil layer (0–10 cm) with a stainless-steel hand shovel and homogenized after removing large roots and stones to obtain a composite bulk sample of about 1 kg for each sampling point. The plants used for feed were collected from each corner and the middle of the plots and combined to make a composite sample. The milk samples were collected during the morning milking session in chemical-free glass bottles and stored frozen (–20 °C) until the analysis.

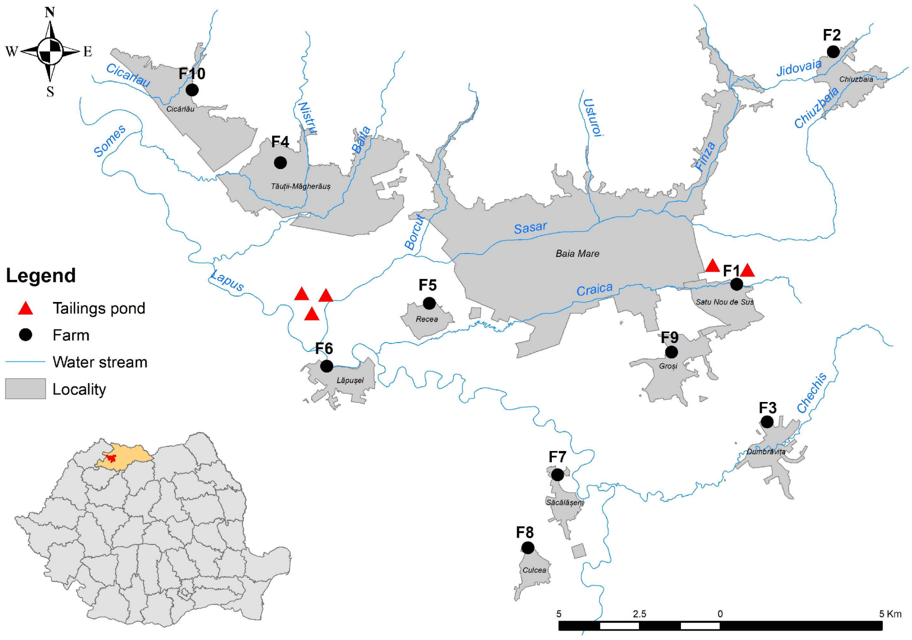


Figure 1. Study area.

2.2. Sample Preparation and Analysis

The water samples were liquid–liquid extracted with hexane, purified, and concentrated according to the Standard ISO 6468:1996 [27,28]. The soil and feed samples were dried at room temperature, then subjected to ultrasound-assisted extraction according to EPA method 3550 [29] using a acetone:hexane (1:1, v/v) mixture and an ultrasonic bath (Bandelin Sonorex, RK 1050, Berlin, Germany). PCB 209 was used as the internal standard and added to the extraction solvents before the extraction process. The extract was preconcentrated using a rotary vacuum evaporator (Laborota 4010, Heidolph Instruments, Schwabach, Germany), purified with Florisil (Merck, Darmstadt, Germany), dried using anhydrous sodium sulphate (Merck, Darmstadt, Germany), and evaporated to near dryness under a nitrogen stream. The residue was redissolved in 1 mL of hexane.

Milk samples were prepared according to the methodology described elsewhere [2]. Briefly, the samples were extracted with a mixture (20:1:5, *v*/*v*) of n-hexane/ethanol/acetonitrile. The extracts were dried on an anhydrous sodium sulphate layer, then evaporated to 5 mL under a nitrogen flux. Then, 1 mL was accurately pipetted into a pre-weighed flask and evaporated to dryness. The lipid content was calculated considering the mass difference. A total of 4 mL extract was purified with Florisil and anhydrous sodium sulphate. The extract eluted with a 1:9 elution mixture (*v*/*v*) of n-hexane/dichloromethane was evaporated to 1.0 mL. All organic solvents used were of gas chromatography grade (Merck, Darmstadt, Germany) and used without additional purification.

A Pesticide–Mix 17 standard solution containing a concentration of 10 µg/mL of each compound in cyclohexane was procured from LGC Standards (Wessel, Germany). The working standard solutions were prepared by the appropriate dilution of the standard solution in dichloromethane. Two certified reference materials (CRMs), namely, BCR–188 (natural milk powder) purchased from LGC Standards (Wessel, Germany) and QC1321 Low-Level Pesticides 1 purchased from Sigma Aldrich (St. Louis, MI, USA) were used for the quality control.

The OCPs studied were hexachlorobenzene (HCB), α-, β-, γ-, δ-, ε-isomers of hexachlorocyclohexane (HCHs), dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD), and dichlorodiphenylchloroethylene (DDE), each with their isomers 4,4′- and 2,4′-, stated as chlorodiphenyl aliphatic compounds or DDTs; aldrin, dieldrin, heptachlor, heptachlor epoxide isomer A, heptachlor epoxide isomer B (stated as cyclodienes), and α- and β-endosulfan.

The concentrations of OCPs were measured using a gas chromatographic system (Agilent Technologies 6890N Santa Clara, CA, USA) equipped with a micro-electron capture detector (GC–ECD) and capillary column with a 30 mL × 0.32 mm ID × 3.0 µm film thickness, DB–1 (Agilent J&W, Santa Clara, CA, USA). The random confirmation of OCPs was performed using a GC (6890N, Agilent Technologies, Santa Clara, CA, USA) coupled with a mass spectrometer (5973N MSD, Agilent Technologies, Santa Clara, CA, USA) on a DP–5 capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness), in the selected ion-monitoring (SIM) mode. The electron-impact ionization of 70 eV and a solvent delay of 6 min were used for the mass spectrometer acquisition. For the quantification, two or three specific ions (the most abundant as a quantifier ion and one or two ions for confirmation) were monitored for each compound (Table 1). For the soil and feed samples, the results were determined based on the dry matter content.

Table 1. GC-MS retention times and selected ions for MS detection in SIM mode.

Compound	Retention Time, min	Monitor Ions, *m*/*z* [1]	Linearity (r^2)
Hexachlorobenzene (HCB)	30.696	284/249, 286	0.9998
α-HCH	30.383	181/219, 109	0.9984
β-HCH	31.805	181/219, 109	0.9997
γ-HCH (lindane)	32.329	181/219, 109	0.9994
δ-HCH	32.064	181/219, 109	0.9992
ε-HCH	33.764	181/219, 109	0.9997
Aldrin	37.100	263/265, 293	0.9994
Dieldrin	43.150	263/79, 345	0.9994
Heptachlor	35.514	272/274, 337	0.9988
Heptachlor epoxide β	35.514	353/81, 263	0.9984
Heptachlor epoxide α	39.211	353/81, 263	0.9994
Endosulfan α	41.779	241/195	0.9998
Endosulfan β	43.752	241/195	0.9989

Table 1. *Cont.*

Compound	Retention Time, min	Monitor Ions, *m*/*z* [1]	Linearity (r^2)
2,4′–DDE	40.481	246/316, 318	0.9999
4,4′–DDE	41.922	246/316, 318	0.9999
2,4′–DDD	42.266	235/165	0.9994
4,4′–DDD	43.865	235/165	0.9993
2,4′–DDT	44.014	235/165, 237	0.9976
4,4′–DDT	45.423	235/165, 237	0.9988

[1] Quantifier ion/1st qualifier ion; [2] nd qualifier ion.

3. Results and Discussion

3.1. Quality Control and Assurance

A control sample (solvents and reagents prepared throughout the same analytical procedure) was used to verify the potential cross-contamination and interference after every 10th sample. No OCP was detected in the control sample. The limits of detection (LOD) and limits of quantification (LOQ) calculated as signal-to-noise ratio of 3:1 and 3:10, respectively, were measured at the approximate retention time of the corresponding analyte peak. The LOQ was roughly considered as 1.0 µg pesticide/L of solvent and was confirmed by spiking the soil, feed, milk, and water with the concentrations of 0.05 µg/kg of dry soil, 0.05 µg/kg of dry feed, 0.05 ng/g of fat raw milk, and 0.001 µg/L of water, respectively, by adding 1 mL of the Pesticide–Mix 17 standard solution containing a concentration of 1.0 µg/L of solvent of each compound, in each matrix sample. The average recovery values were in the range of 71.5 to 118.3%, with the relative standard deviations (RSDs) of six parallel samples lower than 15.5% for all the OCPs and investigated matrices (Table 2). The recovery rates of the internal standard PCB209 ranged between 75.2 and 101.6%. The analyzed concentrations of all OCPs were in good agreement with the certified concentrations in the cases of both CRMs (Table 3).

Table 2. Average (n = 6) recoveries (%) and relative standard deviation (RSD, %) of OCPs in fortified matrices of water (0.0020/0.0010 µg/L), soil (20/0.050 µg/kg), feed (20/0.050 µg/kg), and milk (20/0.050 µg/kg).

Compound	Water 0.002/0.001 µg/L		Soil 20/0.050 µg/kg		Feed 20/0.050 µg/kg		Milk 20/0.050 ng/g Fat	
	Recovery	**RSD**	**Recovery**	**RSD**	**Recovery**	**RSD**	**Recovery**	**RSD**
Hexachlorobenzene	88/85	2.3/2.0	97/86	6.8/5.2	104/95	3.5/4.4	93/91	8.7/9.0
α-HCH	96/91	3.1/2.2	81/80	11.2/6.2	86/79	9.4/4.3	88/86	13.0/6.0
β-HCH	101/95	4.4/3.6	86/85	7.9/6.3	80/91	6.9/5.8	82/79	5.0/9.0
γ-HCH (lindane)	91/95	1.2/2.0	88/81	5.5/3.2	94/88	4.9/6.2	91/87	4.3/8.9
δ-HCH	116/98	1.9/0.9	93/91	4.9/7.2	90/87	5.1/8.0	96/111	6.1/6.0
ε-HCH	103/105	0.8/1.6	96/91	3.5/4.0	88/81	4.4/4.6	86/77	5.0/8.4
Aldrin	86/90	2.2/3.0	81/80	2.9/2.7	90/79	3.0/3.6	81/77	4.4/6.1
Dieldrin	105/112	1.6/0.9	95/82	4.4/6.1	79/89	11.8/7.9	85/80	8.2/6.7
Heptachlor	85/105	5.1/2.0	90/86	6.5/4.8	118/93	4.4/6.3	105/86	15.5/12.4
Heptachlor epoxide β	89/93	0.7/1.8	81/75	7.0/8.8	107/86	1.9/5.3	83/89	10.0/6.7
Heptachlorepoxide α	88/93	1.8/1.5	85/86	3.1/4.0	104/91	3.3/6.2	91/79	7.4/5.6
Endosulfan β	109/99	2.5/3.0	99/85	4.7/3.5	87/104	6.8/7.0	93/80	5.7/5.5
Endosulfan α	95/92	2.0/1.5	88/85	4.5/5.1	91/87	2.9/7.2	86/77	5.9/9.9
2,4′–DDE	82/91	3.0/5.2	79/83	5.0/3.9	87/88	3.8/2.2	74/76	6.1/15.1
4,4′–DDE	79/81	1.6/4.8	72/77	6.1/7.1	106/82	3.1/4.4	82/76	5.2/12.0
2,4′–DDD	101/105	0.9/2.2	92/90	2.8/3.5	97/97	6.1/4.6	93/88	3.8/3.3
4,4′–DDD	72/80	3.1/2.8	77/79	4.0/5.1	81/84	8.4/9.1	75/76	8.1/11.6
2,4′–DDT	91/89	1.0/1.6	86/77	5.2/4.3	88/81	7.2/4.7	81/76	5.6/9.2
4,4′–DDT	86/93	2.2/1.1	81/75	3.6/4.0	90/83	3.2/5.1	83/79	4.8/6.1
PCB209 (internal standard)	99/95	1.8/0.6	102/91	11.2/5.8	75/77	9.8/3.8	86/81	7.9/2.9

Table 3. Recoveries of organochlorine pesticides using CRMs of milk and water (n = 3).

Compound	BCR-188 (Milk)				QC1321 (Water)			
	Certified, μg/kg	Measured, μg/kg,	RSD, %	Recovery, %	Certified, μg/L	Measured, μg/L	RSD, %	Recovery, %
Hexachlorobenzene	37.4	36.1	6.2	97	-	-	-	-
β-HCH	12.0	10.1	9.3	84	-	-	-	-
4,4′–DDE	51.0	44.0	10.6	86	5.74	5.63	9.50	98
Dieldrin	36.1	38.1	5.1	105	5.91	6.32	3.90	107
4,4′–DDT	69.0	62.2	9.6	90	2.18	2.35	11.8	108
Aldrin	-	-	-	-	6.04	6.83	4.10	113
4,4′–DDD	-	-	-	-	7.29	6.12	10.9	84
Heptachlor	-	-	-	-	2.34	2.18	4.00	93
Heptachlor epoxide α	-	-	-	-	4.98	5.13	6.70	103

3.2. Organochlorine Pesticides in Soil

OCPs can be retained in soil, thus increasing the chances of high persistence in the environment [11]. In this study, it was considered that cows ingested soil directly during grazing, and this should be taken into consideration due to the high contamination of some arable fields in rural areas, as well as fields impacted by the industry and intensive agriculture [30,31]. The range, average, and standard deviation (SD) of OCPs in the soil samples in μg/kg of dry weight (dw) are presented in Table 4, and the contribution of individual OCPs to the total OCPs in the soil samples are displayed in Figure 2.

Table 4. OCP concentrations (μg/kg) in soil (n = 10), normal values in soil (NV), and alert level (AL) for sensitive soil uses.

Compound	Min.–Max. (μg/kg)	Average	SD	NV	AL [31]
Hexachlorobenzene (HCB)	<0.05–0.21	0.08	0.06	–	–
α-HCH	0.38–1.9	0.89	0.49	<2	10
β-HCH	<0.05–4.1	1.2	1.3	<1	50
γ-HCH (lindane)	<0.05–1.1	0.50	0.31	<1	20
δ-HCH	0.25–4.5	0.81	1.3	<1	50
ε-HCH	<0.05–5.0	1.25	1.69	–	–
Σ HCHs	1.3–15	4.6	4.1	<5	250
Aldrin	<0.05–1.2	0.38	0.41	–	–
Dieldrin	<0.05–1.6	0.41	0.51	–	–
Heptachlor	<0.05–1.3	0.65	0.44	–	–
Heptachlor epoxide β	0.18–1.2	0.53	0.38	–	–
Heptachlor epoxide α	<0.05–0.40	0.21	0.10	–	–
Endosulfan β	<0.05–0.51	0.22	0.17	–	–
Endosulfan α	<0.05–0.43	0.07	0.13	–	–
2,4′–DDE	0.08–1.6	0.57	0.58	–	–
4,4′–DDE	0.18–13	3.6	4.5	–	–
Σ DDEs	0.31–14	4.1	4.8	<50	250
2,4′–DDD	<0.05–3.6	0.77	1.13	–	–
4,4′–DDD	<0.05–16	3.5	5.8	–	–
Σ DDDs	0.16–19	4.2	6.9	<50	250
2,4′–DDT	<0.05–2.1	0.48	0.84	–	–
4,4′–DDT	<0.05–40	7.7	15	–	–
Σ DDTs	0.05–41.8	8.1	16	<50	250
Total DDTs [1]	0.52–66	17	25	<150	250
Total OCPs	3.4–76	24	26	<200	1000

[1] Σ(2,4′–DDE + 4,4′–DDE + 2,4′–DDD + 4,4′–DDD + 2,4′–DDT + 4,4′–DDT).

OCPs were measured in all soil samples, the Σ HCHs being the dominant compounds in soils from F1, F2, F3, F8, and F10, whereas the Σ DDTs were the dominant compounds in F4–F7. Generally, the other pesticides represented less than 20% of the total OCP concentration, except for soils in F1, F2, and F8, where it represented about half of the total OCP concentration. For the statistical analysis, the concentrations below the LOQ were considered half of the LOQ value (0.025 µg/kg) [32]. Concentrations of individual and total OCPs were, generally, around the normal values specified by the national legislation and were much lower than the alert levels (ALs) set for sensitive soil uses [33]. Total HCH, ΣDDE, ΣDDD, and ΣDDT are presented in Figure 3. The maximum concentration of the different OCP classes was measured in different soil samples. Thus, the highest Σ HCH level was measured in the soil from F3, while the lowest was in F7, whereas the highest Σ DDEs in soils were from F7 and the lowest in F2. The concentrations of both Σ DDDs and Σ DDTs were generally low, except for soil samples F4 and F5, where they were much higher, but without exceeding the corresponding ALs.

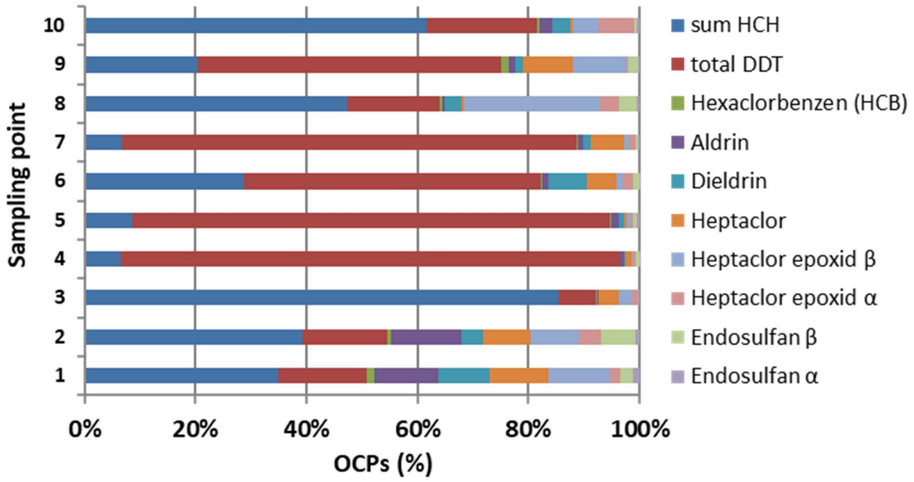
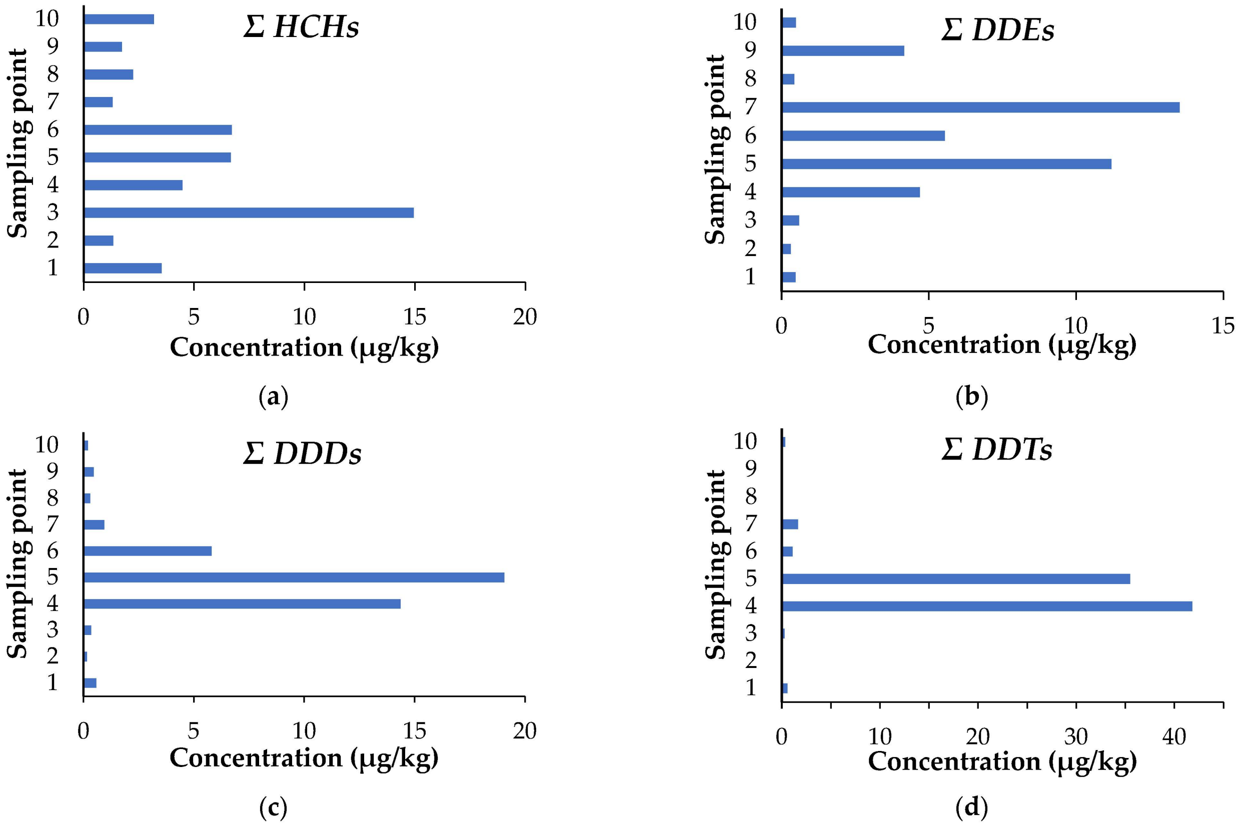


Figure 2. Share of individual OCPs in total OCP concentrations in soil.

HCB was detected in 50% of the analyzed samples, with a maximum value of 0.21 µg/kg in F5. Relatively low concentrations of endosulfans were determined in 80% of the samples. The α-endosulfan concentration was lower than that of the β-isomer due to the higher degradation rate of the α-isomer than of β. Compounds of HCHs, cyclodienes, and endosulfans classes were detected in all the analyzed samples, in low concentrations, indicating the absence of their recent applications, and also their ubiquity in the study area. The ratios of α-HCH and γ-HCH indicate the source of HCH. A ratio between 4 and 7 shows that the source is a technical HCH, and if the ratio is near zero, the source is lindane [34]. The high values in the present study (average 4.99) indicated technical HCH as the source of HCH. In the analyzed soil samples, the frequencies of α-HCH and δ-HCH were 100%, whereas the frequencies of the other three HCH isomers were 90%.

Chlorodifenyl aliphatic compounds were detected in all the analyzed soil samples at higher concentrations than those of the other OCPs. The highest concentrations were found in F5 (65.7 µg/kg) and F4 (60.9 µg/kg); the dominant compound was 4,4′–DDT. The sum of DDTs was in the range of 0.52–65.7 µg/kg, with an average of 6.5 µg/kg, and were similar to those reported by Westbom et al. in soils from Ethiopia [35], but lower than in the soils from the Pearl River Delta in China (average: 37.6 µg/kg) [36], in agricultural soils in Central China (average: 151.6 µg/kg) [37] and Argentina (26.3 µg/kg), [38] and much lower than in the surface soil from rural areas with extensive historical agricultural pesticide use in the Republic of Tajikistan (thousands of µg/kg) [39]. The concentrations of lindane, β–HCH, dieldrin, and 4,4′-DDT were 10–100-times lower than those measured in soils collected from cocoa-growing areas in Ghana [40]. However, the concentrations of DDTs, 4,4′–DDE, 2,4′–DDD, β–HCH, α–HCH, γ–HCH, and δ–HCH were comparable with those from the soils from Benevento, Italy [11].

DDT is biodegraded by microorganisms to DDE and DDD in aerobic and anaerobic conditions, respectively; thus, the DDT/(DDE + DDD) ratio differentiates between recent and historical inputs of DDTs [41]. A low ratio indicates the legacy pollution of DDT and a high ratio indicates recent inputs of DDT. The DDT/(DDE + DDD) ratio was in the range of 0.01–2.20, with an average value of 0.50. Generally, the ratio was sub-unitary, indicating no recent DDT inputs, but a continuous, historical exposure. In farms F4 (2.20) and F5 (1.17), the ratio was above 1, suggesting the recent use of DDTs, although their use was prohibited [42].



(**a**) (**b**) (**c**) (**d**)

Figure 3. Average concentrations of (**a**) total HCHs; (**b**) Σ DDEs; (**c**) Σ DDDs; (**d**) Σ DDTs (µg/kg) in soils.

3.3. Organochlorine Pesticides in Water

OCPs were detected in all water samples, the lowest concentrations found in water from F1 (0.0187 µg/L) and the highest in F10 (0.0465 µg/L). The highest average concentrations were recorded for endosulfan α, followed by endosulfan β, 2,4′–DDT, δ–HCH, and ε–HCH. The range, averages, and standard deviations of OCPs in water samples are presented in Table 5. Hexachlorobenzene was detected in only two of the analyzed samples, with a maximum value of 0.0022 µg/L in water collected from F5. Among the HCH isomers, δ–HCH had the highest concentrations in the water source of F6 (0.0058 µg/L) and 0.0057 µg/L in farms F3 and F4. The ε–HCH isomer and aldrin were detected in 7 and 8 of the 10 analyzed water samples, respectively. Dieldrin, heptachlor, heptachlor epoxide β, and endosulfan α were not detected in any investigated sample. Among the chlorodifenyl aliphatic compounds, 2,4′–DDT and 2,4′–DDE were detected in 6 of the 10 samples, whereas 4,4′–DDE was detected in 2 water samples.

The individual OCP concentrations were lower than their corresponding maximum admitted concentrations (MACs) set by the Romanian legislation, with two orders of magnitude [43]. The total OCPs were lower than the MAC with one order of magnitude. For the statistical analysis, the concentrations lower than the LOQ were considered half of the LOQ value.

The concentrations of lindane, endosulfan α, dieldrin, and 4,4′–DDT were an order of magnitude lower than the drinking water samples from cocoa-growing areas in Ghana [40] and considerably lower (by three orders of magnitude) than in the drinking water in Yucatan, Mexico [44]. Total OCP concentrations were comparable with the sum of OCPs in the Beiluo River, Loess Plateau, China, used as drinking and household water [45], and lower than in the Yangtze River, used as a drinking water source [46].

Table 5. OCP concentrations (µg/L) in water samples (n = 10) and maximum admitted concentration (MAC).

Compound	Min.–Max.	Average	SD	MAC [42]
Hexachlorobenzene (HCB)	<0.001–0.002	0.001	0.001	0.1
α-HCH	<0.001–0.003	0.001	0.001	0.1
β-HCH	<0.001–0.006	0.002	0.002	0.1
γ-HCH (lindane)	<0.001–0.002	0.001	0.000	0.1
δ-HCH	<0.001–0.006	0.003	0.003	0.1
ε-HCH	<0.001–0.008	0.002	0.002	0.1
Aldrin	<0.001–0.004	0.002	0.001	0.03
Dieldrin	<0.001	<0.001	0.000	0.03
Heptachlor	<0.001	<0.001	0.000	0.1
Heptachlor epoxide β	<0.0010	<0.001	0.000	0.03
Heptachlor epoxide α	<0.001–0.002	0.001	0.001	0.03
Endosulfan α	<0.009	<0.009	0.000	0.1
Endosulfan β	<0.005–0.010	0.004	0.003	0.1
2,4′–DDE	<0.001–0.009	0.002	0.003	0.1
4,4′–DDE	<0.005–0.004	0.001	0.001	0.1
2,4′–DDD	<0.001–0.003	0.002	0.001	0.1
4,4′–DDD	<0.001–0.002	0.001	0.001	0.1
2,4′–DDT	<0.001–0.003	0.001	0.001	0.1
4,4′–DDT	<0.001–0.006	0.002	0.002	0.1
Total OCPs	0.019–0.046	0.032	0.008	0.5

3.4. Organochlorine Pesticides in Feed

OCPs were determined in all feed samples, the main compounds being dichlorodiphenyltrichloroethanes (DDTs). The range of variation, average values, and standard deviations of OCPs in the investigated feed samples are presented in Table 6. The values obtained are expressed in µg/kg dw. In the statistical calculations, concentrations lower than the LOQ (<0.05 µg/kg dw) were considered half of this value.

Ruminants, such cows, can contribute to the OCPs' transfer into the human food chain by intaking large amounts of herbage or silage in a short period [47]. Like any organism, animals' health, as well as animal product quality, depends on the quality of the environment, water, and food [48]. Thus, animal feed must not only meet the nutritional needs of the animals to ensure their welfare, but must also have no or a limited content of toxic compounds. In the food chain, feed quality is of the utmost importance, as it can affect two target populations: first, the animals that consume it, and secondly, the human consumers of animal-originated food. The maximum admitted concentrations of toxic contaminants in animal feed are regulated by Directive 2002/32/EC as the maximum residue level (MRL) [17].

The individual concentrations and their sum were lower than the MRL values provided by the European legislation [17]. Low concentrations of hexachlorobenzene (HCB) were identified in four of the ten analyzed feed samples at low concentrations, with a mean value of 0.05 µg/kg. HCH compounds were detected in all the analyzed samples, except in the sample from F2. The average concentrations varied as follows: ε–HCH > α–HCH > β–HCH > δ–HCH > γ–HCH. Total concentrations of HCHs (Σ α–, β–, γ–, δ–, ε–HCH isomers) varied between 0.13 µg/kg (F2) and 24.5 µg/kg (F3), with an average of 4.67 µg/kg and standard deviation of 7.47 µg/kg. Isomers α- and/or β-endosulfan were detected in 60% of the samples, in low concentrations (<0.05–0.55 µg/kg). Cyclodiene compounds were determined in all the feed samples in low concentrations, the average concentrations decreasing in the following order: heptachlor epoxide β > aldrin >

heptachlor > dieldrin > heptachlor epoxide α. Chlorodiphenyl aliphatic compounds were detected in all the feed samples, the highest concentrations being measured in samples from F5 (3.61 µg/kg) and F9 (3.27 µg/kg). The dominant chlorodiphenyl aliphatic compound was 4,4′–DDE. The total DDT concentrations varied in the range of 0.20–3.61 µg/kg, with an average of 1.36 µg/kg. The OCP concentrations were relatively low, indicating no recent input but their ubiquity in the investigated area. The concentrations of α-HCH, β-HCH, aldrin, dieldrin, and heptachlor in our feed samples were lower than those reported by Panseri et al. 2013 [49] and Deka et al. 2004 [50], and were far below the MRL values stated by the EU.

Table 6. OCP concentrations (µg/kg) in feed samples (n = 10) and maximum residue level (MRL).

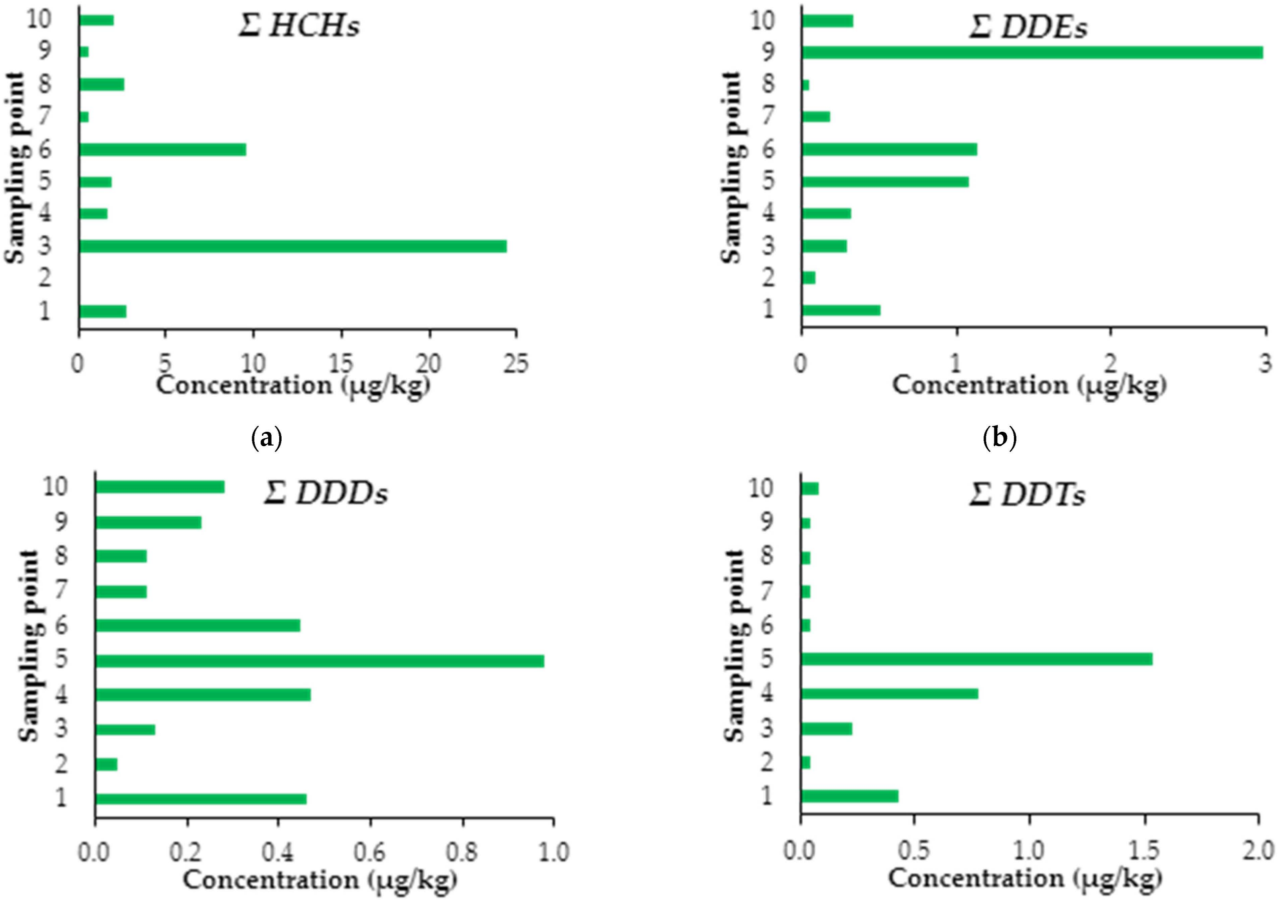
Compound	Min.–Max.	Average	SD	MRL [16]
Hexachlorobenzene (HCB)	<0.05–0.12	0.05	0.04	10
α-HCH	<0.05–4.3	0.96	1.3	20
β-HCH	<0.05–3.67	0.90	1.32	10
γ-HCH (lindane)	<0.05–0.67	0.25	0.21	200
δ-HCH	<0.05–5.1	0.60	1.6	–
ε-HCH	<0.05–11	2.0	3.7	–
Aldrin	<0.05–1.7	0.41	0.52	10
Dieldrin	<0.05–0.84	0.18	0.29	10
Heptachlor	<0.05–1.1	0.40	0.37	–
Heptachlor epoxide β	<0.05–1.5	0.59	0.48	–
Heptachlor epoxide α	<0.05–0.46	0.10	0.14	–
Heptachlor total [1]	<0.17–1.5	0.69	0.46	10
Endosulfan α	<0.05–0.18	0.07	0.06	–
Endosulfan β	<0.05–0.37	0.12	0.15	–
Σ Endosulfan [2]	<0.05–0.55	0.19	0.17	100
2,4′–DDE	<0.05–0.94	0.27	0.31	–
4,4′–DDE	<0.05–2.9	0.42	0.86	–
2,4′–DDD	<0.05–0.63	0.21	0.21	–
4,4′–DDD	<0.05–0.35	0.12	0.11	–
2,4′–DDT	<0.05–0.88	0.19	0.29	–
4,4′–DDT	<0.05–0.66	0.14	0.21	–
Total DDTs [3]	<0.20–3.6	1.4	1.2	50
Total OCPs	0.97–25	7.0	7.7	–

[1] Heptachlor + heptachlor epoxide α + heptachlor epoxide β; [2] endosulfan α + endosulfan β; [3] 2,4′–DDE + 4,4′–DDE + 2,4′–DDD + 4,4′–DDD + 2,4′–DDT + 4,4′–DDT.

The concentrations of Σ HCHs, Σ DDEs, Σ DDDs, and Σ DDTs (Figure 4) varied from sample to sample. The highest concentration of HCH was measured in the feed from F3, whereas in the other feed samples, it was much lower. The highest concentration of DDE was found in the feed from F9, of DDDs in F5, and DDTs in F5.

3.5. Organochlorine Pesticides in Milk

Organochlorine pesticides were detected in every milk sample. Concentrations of OCPs in milk were reported as ng/g fat. The fat content of milk varied between the range of 3.8–4.2%, average: 4.0 ± 0.15%. The ranges, averages, and standard deviations in the milk samples are presented in Table 7. For the statistical analysis, concentrations lower than the LOQ (0.05 ng/g fat) were considered half of the LOQ (0.025 ng/g fat) [32]. In order to compare the obtained OCP concentrations in the milk samples, the units of measure were converted from ng/g fat (as shown in the present study) to µg/kg (as set by the Order 23/2007 [51] and Regulation 396/2005 [52]); thus, the obtained concentrations were multiplied by 0.04, as the average fat concentration in the milk samples was 4% [53], and we assumed a density of 1.0 g/mL for milk [54].



(**a**) (**b**)

(**c**) (**d**)

Figure 4. Average concentrations of (**a**) total HCHs; (**b**) Σ DDEs; (**c**) Σ DDDs; (**d**) Σ DDTs (µg/kg) in feed.

The β–HCH isomer and 4,4′–DDE were detected in all the milk samples. The OCP concentrations, both individual and their sums, in the milk were below the maximum residue levels specified by the European [51,55] and national [52] legislations by 20–90 times. HCB was detected in nine milk samples, with maximum values in the sample from F5 (1.4 ng/g fat). α– and β– endosulfans and were detected in 8 and 7 samples, respectively in low concentrations. HCH compounds were also detected in all the milk samples. The mean concentrations of the HCH isomers decreased in the following order: β–HCH > γ–HCH > δ–HCH > α–HCH > ε–HCH. Low cyclodiene compound concentrations were detected in 90% of the samples. Their average concentrations varied as follows: heptachlor > dieldrin > heptachlor epoxide β >aldrin >heptachlor epoxide α. Chlorodiphenyl aliphatic compounds were detected in all milk samples, the highest sum of six DDT isomers being measured in the sample from F9 (18.1 ng/g fat) and the lowest in the sample from F8 (1.2 ng/g fat). Σ HCHs, Σ DDEs, Σ DDDs, and Σ DDTs (Figure 5) varied from sample to sample. The highest concentration of HCH was measured in the milk from F8, the highest concentration of DDE in the milk from F9, of DDD in F1, and DDT in F7.

Similar to the feed samples, the dominant compound was 4,4′–DDE. The aldrin, dieldrin, lindane, endosulfan, 4,4′–DDE, 4,4′–DDT, and 2,4′–DDT concentrations were much lower (by 5–50 times) than those reported in raw and pasteurized milk samples from Kampala markets, Uganda [56], in milk samples from different regions in Ethiopia [57], and from local markets in Sahiwal and Lahore, Pakistan [58,59]. The 4,4′–DDE and 4,4′–DDT concentrations were approximately two-times lower than in cow milk samples from Dhaka markets, Bangladesh [60].

Table 7. OCP concentrations (ng/g fat) in milk samples (n = 10) and maximum residue level (MRL).

Compound	Min.–Max.	Average	SD	MRL [51,52,55] (µg/kg/ng/g Fat)
Hexachlorobenzene (HCB)	<0.05–1.4	0.62	0.40	5/125 [2]
α-HCH	<0.05–3.1	1.1	0.94	10/250
β-HCH	0.28–12	4.7	4.0	10/250
γ-HCH (lindane)	<0.05–6.9	1.9	2.0	10/250
δ-HCH	<0.05–5.1	1.4	1.5	–
ε-HCH	<0.05–1.1	0.44	0.44	–
Aldrin	<0.05–1.5	0.60	0.53	–
Dieldrin	<0.05–3.1	1.0	1.1	–
Aldrin + Dieldrin, expressed as aldrin	0.11–4.0	2.3	1.3	6/150
Heptachlor	<0.05–5.0	1.7	1.7	–
Heptachlor epoxide β	<0.05–1.9	0.91	0.63	–
Heptachlor epoxide α	<0.05–0.45	0.18	0.15	–
Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	0.07–5.7	3.1	1.7	4/100
Endosulfan β	<0.05–1.5	0.43	0.42	–
Endosulfan α	<0.05–1.3	0.40	0.37	–
Σ Endosulfan	<0.05–1.9	0.84	0.67	4/100 [2]
2,4′–DDE	<0.05–0.14	0.07	0.04	–
4,4′–DDE	0.61–18	4.4	5.2	–
2,4′–DDD	<0.05–0.46	0.12	0.14	–
4,4′–DDD	<0.05–0.47	0.13	0.13	–
2,4′–DDT	<0.05–0.23	0.12	0.07	–
4,4′–DDT	<0.05–3.2	0.52	0.96	–
Total DDTs [1]	1.2–18.1	5.2	5.2	40/1000

[1] 4,4′–DDE + 4,4′–DDD + 2,4′–DDT + 4,4′–DDT; [2] values in bold are expressed as ng/g fat.

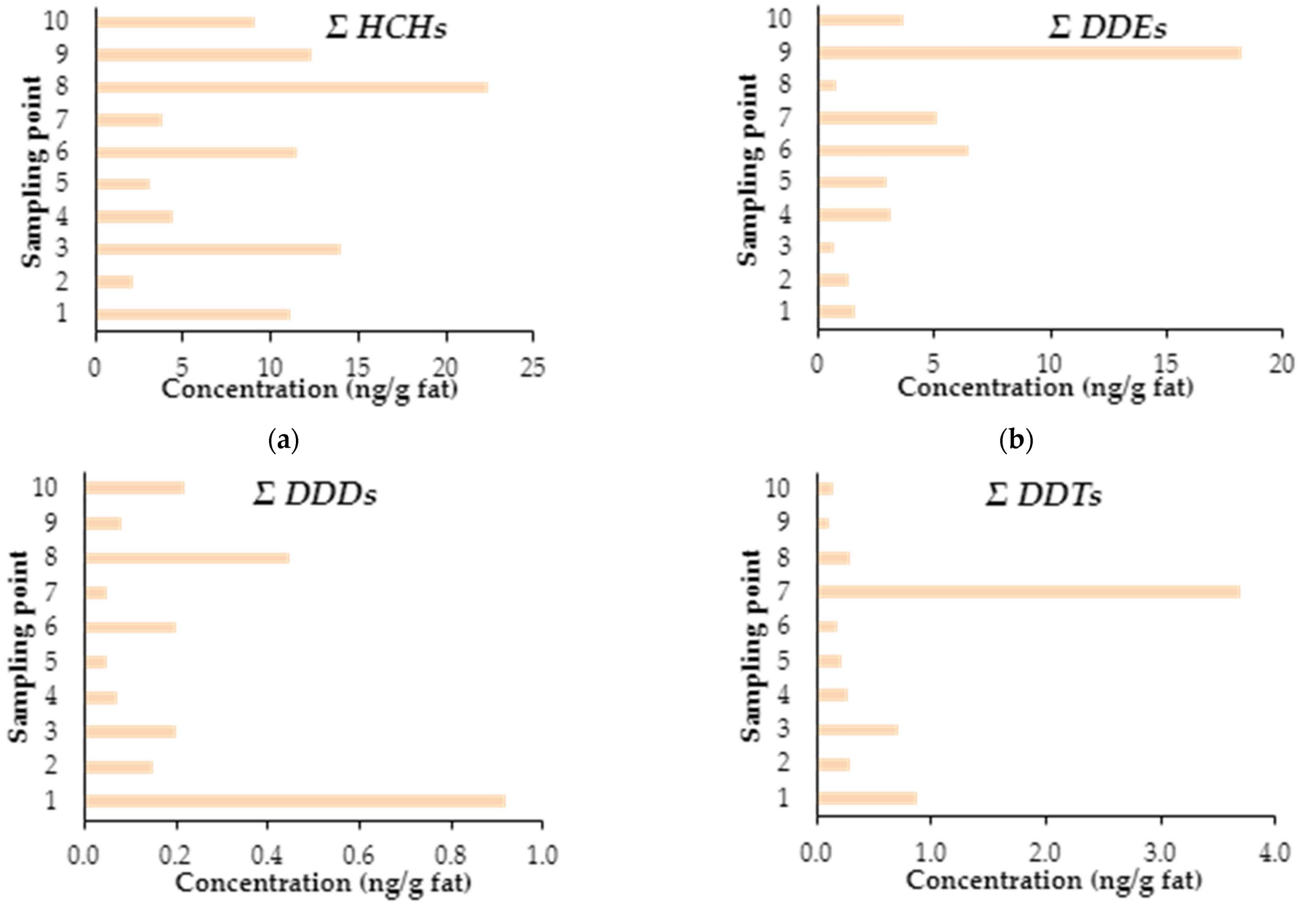
In all the investigated matrices, the predominant compounds were HCH and DDT isomers, with 4,4′–DDE as the main metabolite, except in the soil where 4,4′–DDT was the most prominent compound, and the concentrations of HCB, cyclodienes, and endosulfans were low, accounting for less the 20% of the total OCP concentration. The mean ΣHCHs in milk was 4 times higher than in the soil and feed, respectively, and the mean ΣDDTs in milk were 4-times higher and 1.5-times lower than in the feed and soil, respectively. In the feed, water, and soil samples, the main isomers of HCH were δ-, ε-, and β-HCH, and in milk, the main isomer was β-HCH, showing the highest BTF value among HCH isomers, with two super-unit values. Similarly, the BTF value for the 4,4′–DDE isomer showed the highest BTF value among the DDT isomers.

3.6. OCP Carryover Rate and Biotransfer Factor

Individual concentrations of OCPs in soil, feed, milk, and water were used to calculate the OCP carryover rate (COR, Equation (1)) and the biotransfer factor ($BTF_{feed–milk}$, Equation (2)). Under steady-state conditions, the COR describes the fraction of ingested OCPs transferred to animal tissue [61,62]. Generally, the COR is not strongly influenced by the animal's lactation rate, body weight, fat, or diet [46].

$$COR = C_{milk} \times M_y \times 100/(C_{feed} \times I_{feed} + C_{water} \times I_{water} + C_{soil} \times I_{soil}) \quad (1)$$

where COR is the carryover rate (%); C_{milk} is the concentration of OCPs in milk (µg/kg); M_y is the milking yield (kg/day); C_{feed}, C_{water}, and C_{soil} are the OCP concentrations in the feed (mg/kg dw), water (µg/L), and soil (mg/kg dw), respectively; and I_{feed}, I_{water}, and I_{soil} are the daily intake values of the feed, water, and soil (kg/day dw). Based on the average milk production in the investigated farms, an M_y value of 20 kg/day was used for the calculations.



(**a**) (**b**)

(**c**) (**d**)

Figure 5. Average concentrations of (**a**) total HCHs; (**b**) Σ DDEs; (**c**) Σ DDDs; (**d**) Σ DDTs (ng/g fat) in milk.

The calculation of the COR was based on the following factors: (i) during the study, the intake was constant and equal to 20 kg/day of dw feed [33], 20 L/day of water [63], and 0.41 kg/day of dw soil [30,31]; (ii) the animal diet was represented exclusively by the grass/feed harvested from the investigated pastures, water from the analyzed source, and soil from the investigated pastures; and (iii) the concentrations of the contaminants represented the determined concentrations in the water, soil, and feed, considering that equilibrium conditions were achieved. For the calculation of the COR, the concentrations of OCPs in the milk were converted from ng/g fat to µg/kg (milk), considering the milk fat content of 4% [51]. Concentrations of OCPs in the milk, soil, and feed <LOQ were considered equal to LOQ/2 = 0.025 (ng/g fat).

The BTF quantifies the dietary transfer of environmental contaminants into the animal and human body. The BTFs of OCPs from vegetation to milk were calculated as the ratio between the OCP concentration in milk and the daily intake of OCPs, according to Equation (2) [64,65]:

$$BTF = C_{milk}/D \tag{2}$$

where C_{milk} is the concentration of the contaminant (OCP) in milk (µg/kg ww) and D (Equation (3)) is the dietary intake of OCPs (µg/day). The concentration of OCPs in milk was converted from ng/g fat into µg/kg whole milk.

$$D = C_{feed} \times I_{feed} \tag{3}$$

where C_{feed} is the concentration of OCPs in the feed (mg/kg dw) and I_{feed} is the daily intake of feed (20 kg/day dw). For the BTF calculation, OCP concentrations <LOQ in milk and feed were considered equal to LOQ/2 = 0.025 (ng/g fat).

The calculation of the BTF was based on the following factors: during the study, feed/fodder intake was constant and equal to 20 kg dw/day for each case [32], and we assumed that animal diet was represented exclusively by the grass/vegetation harvested from the investigated pastures.

Basic statistics (minimum, maximum, averages, and standard deviations) of the COR and BTF are presented in Table 8. The COR values varied over four orders of magnitudes, from 0.10% (2,4′–DDE) to 250% (4,4′–DDT). The high variability of the COR could be a consequence of the diverse structure of OCPs, which caused varying animal behavior in the environment.

Table 8. Basic statistic of carryover (COR) for OCPs in feed, water, and soil to milk and of biotransfer factors ($BTF_{feed–milk}$) for organochlorine compounds from the feed to milk.

Compound	**COR, %**			**$BTF_{feed–milk}$**		
	Min–Max	**Average**	**Std. Dev**	**Min–Max**	**Average**	**Std. Dev**
Hexachlorobenzene (HCB)	3.8–120	56	38	0.0080–0.416	0.147	0.139
α–HCH	0.30–182	31	57	0.0007–0.286	0.044	0.090
β–HCH	7.3–115	55	37	0.0013–1.016	0.312	0.425
γ–HCH (Lindane)	0.18–121	46	43	0.0007–0.816	0.115	0.250
δ–HCH	2.3–88	41	33	0.0003–0.294	0.081	0.093
ε–HCH	0.20–46	7.3	14	0.0001–0.133	0.028	0.053
Aldrin	0.15–210	45	83	0.0001–0.180	0.034	0.063
Dieldrin	2.0–123	52	46	0.0022–0.204	0.087	0.084
Heptachlor	2.0–88	24	27	0.0019–0.302	0.051	0.097
Heptachlor epoxide β	0.69–30	10	10	0.0015–0.041	0.008	0.012
Heptachlor epoxide α	0.21–63	20	22	0.0001–0.034	0.006	0.010
Endosulfan α	0.68–133	42	42	0.0003–0.072	0.021	0.029
Endosulfan β	0.27–162	38	53	0.0001–0.059	0.016	0.021
2,4′–DDE	0.10–19	4.4	7.2	0.0002–0.020	0.005	0.007
4,4′–DDE	22–75	49	21	0.0109–1.408	0.334	0.453
2,4′–DDD	0.14–31	7.9	11	0.0001–0.024	0.004	0.008
4,4′–DDD	0.15–71	11	22	0.0001–0.020	0.003	0.006
2,4′–DDT	0.22–32	12	11	0.0004–0.016	0.007	0.006
4,4′–DDT	0.12–250	43	81	0.0002–0.294	0.039	0.091

The BTF values varied widely due to the very diverse chemical structure of the organochlorine compounds, which implied major differences in their metabolism and biotransformation, storage in various organs, and excretion [66]. Similar to the CORs, the values of BTFs varied largely, from 0.000075 for aldrin to 1.408 for 4,4′–DDE. Additionally, the values higher than the unit for some BTFs could be due to the animal body accumulation and biomagnification factors of these organochlorine compounds.

3.7. Cows' Dietary Exposure to Soil, Water, and Feed Ingestion

The accumulation of toxic organic compounds in agricultural soils is of great importance due to their negative impact, in general, and of OCPs in particular, on cultivated plants, the health of the animals that consume them, and the soil ecosystem [31].

According to the European Food Safety Authority (EFSA), dietary exposure, expressed as (kg/day)/body weight (kg), is estimated as the concentration of chemicals in feedstuff multiplied by the amount of feedstuff consumed, divided by body weight [67]. Thus, the dietary exposure of the cows was calculated by a deterministic method for chronic exposure to low concentrations, namely, by calculating the estimated daily intake (EDI; Equation (4)) for each contaminant, considering the three components of ingestion: feed, water, and soil consumption. As suggested by the EFSA, the body weight (bw) of a lactating cow is 650 kg, used as the default value [67].

$$EDI = (C_{feed} \times I_{feed} + C_{water} \times I_{water} + C_{soil} \times I_{soil})/bw \quad (4)$$

where EDI is the estimated daily intake of OCPs, in µg/day; C_{feed}, C_{water}, and C_{soil} are the concentrations of OCPs in the feed (mg/kg dw), water (µg/L), and soil (mg/kg dw), respectively; I_{feed} is the daily intake of feed (20 kg/day dw, [33]), I_{water} is the daily intake of water (20 L/day, [63]), I_{soil} is the daily intake of soil (0.41 kg/day dw, [30,31]); and bw is the body weight of a lactating cow (650 kg [67]).

The basic statistics of the EDIs for each OCP calculated by summing the daily intakes of feed, water, and soil in each of the ten sampling points, are presented in Table 9. The EDI values varied between 0.0008 µg/day/bw (for HCB, β-HCH, lindane, and heptachlor) and 0.3509 µg/day/bw (for ε–HCH). In general, the daily intake of OCPs was from feed > daily intake of soil > daily intake of water.

Table 9. Basic statistics for estimated daily intakes (EDIs, µg/day) of organochlorine compounds.

Compound	Min.–Max.	Average	Std. Dev.
Hexachlorobenzene (HCB)	0.0008–0.0039	0.0017	0.0012
α–HCH	0.0011–0.1336	0.0302	0.0392
β–HCH	0.0008–0.1160	0.0284	0.0413
γ–HCH (Lindane)	0.0008–0.0212	0.0081	0.0066
δ–HCH	0.0010–0.1603	0.0190	0.0497
ε–HCH	0.0008–0.3509	0.0611	0.1151
Aldrin	0.0009–0.0519	0.0129	0.0164
Dieldrin	0.0008–0.0265	0.0059	0.0093
Heptachlor	0.0008–0.0340	0.0127	0.0114
Heptachlor epoxide β	0.0009–0.0463	0.0185	0.0149
Heptachlor epoxide α	0.0009–0.0144	0.0033	0.0045
Endosulfan α	0.0009–0.0119	0.0039	0.0048
Endosulfan β	0.0009–0.0058	0.0023	0.0018
2,4′–DDE	0.0009–0.0299	0.0088	0.0097
4,4′–DDE	0.0011–0.0903	0.0154	0.0267
2,4′–DDD	0.0008–0.0217	0.0069	0.0071
4,4′–DDD	0.0008–0.0206	0.0060	0.0067
2,4′–DDT	0.0008–0.0286	0.0062	0.0095
4,4′–DDT	0.0008–0.0414	0.0093	0.0157
Total DDT	0.0066–0.1529	0.0527	0.0482

By summing the individual EDI for each organochlorine compound in each farm, we obtained the total EDI for all organochlorine compounds, which varied between 0.0523 µg/day/bw (F2) and 0.826 µg/day/bw (F3), with an average of 0.261 µg/day/bw ± 0.234 µg/day/bw.

4. Conclusions

Nineteen organochlorine pesticides were determined in the soil, water, feed, and milk from ten small—scale, free—range dairy cow farms in NW Romania. As a step of managing OCP residues in livestock products (milk), we calculated the carryover rate and biotransfer factor after oral exposure via feed, water, and soil. The estimated daily intake (EDI) was also calculated for each contaminant considering the three components of ingestion: feed, water, and soil consumption. Organochlorine compounds did not exceed the maximum permissible concentrations according to the European legislation in any of the studied samples. The HCH and DDT values were the most abundant components in the soil, feed, and milk samples. BTF values higher than the unit suggest the accumulation and biomagnification of OCPs in the animal's body. The EDI values varied between 0.0008 µg/day/bw for HCB, β-HCH, lindane, and heptachlor, and 0.3509 µg/day/bw for ε–HCH. Considering the diffuse contamination of the environment by these compounds and their endocrine-disrupting effects at extremely low concentrations, no potential exposure of the milk-consuming population in the studied area can be considered. The obtained results provide valuable information on the bioaccumulation of OCPs in animals and milk and, thus, the potential human exposure of the human consumer to this food. In the studied farms, constant care results in better environmental management and an increase in the farm's sustainability outcomes. However, the guiding and supervising role of the government promotes the sustainable development of the whole dairy industry chain.

Author Contributions: Conceptualization, M.M., O.C. and E.A.L.; methodology, M.M., O.C. and E.A.L.; investigation, M.M., O.C. and E.A.L.; formal analysis, M.M., E.A.L. and O.C.; writing—original draft preparation, M.M., E.A.L. and O.C.; writing—review and editing, O.C., E.A.L. and M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Saaristo, M.; Brodin, T.; Balshine, S.; Bertram, M.G.; Brooks, B.W.; Ehlman, S.M.; McCallum, E.S.; Sih, A.; Sundin, J.; Wong, B.B.M.; et al. Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. *Proc. Biol. Sci.* **2018**, *285*, 20181297. [CrossRef] [PubMed]
2. Miclean, M.; Cadar, O.; Levei, E. Human health risk assessment of organochlorine compounds associated with raw milk consumption in a Romanian industrial area. *Ital. J. Food Sci.* **2018**, *30*, 1.
3. Marlat, V.L.; Bayen, S.; Castaneda–Cortes, D.; Delbes, G.; Grigorova, P.; Langlois, V.S.; Martyniuk, C.J.; Metcalfe, C.D.; Parent, L.; Rwigemera, A.; et al. Impacts of endocrine disrupting chemicals on reproduction in wildlife and humans. *Environ. Res.* **2022**, *208*, 112584. [CrossRef] [PubMed]
4. Penagos-Tabares, F.; Sulyok, M.; Faas, J.; Krska, R.; Khiaosa-ard, R.; Zebeli, Q. Residues of pesticides and veterinary drugs in diets of dairy cattle from conventional and organic farms in Austria. *Environ. Pollut.* **2023**, *316*, 120626. [CrossRef]
5. Mahmoud, R.; Wainwright, S.R.; Galea, L.A. Sex hormones and adult hippocampal neurogenesis: Regulation, implications, and potential mechanisms. *Front. Neuroendocrinol.* **2016**, *41*, 129–152. [CrossRef] [PubMed]
6. Ferrante, M.C.; Fusco, G.; Monnolo, A.; Saggiomo, F.; Guccione, J.; Mercogliano, R.; Clausi, M.T. Food contamination by PCBs and waste disposal crisis: Evidence from goat milk in Campania (Italy). *Chemosphere* **2017**, *186*, 396–404. [CrossRef] [PubMed]
7. Popa, C.L.; Dontu, S.I.; Levei, E.A.; Ioja, C.I.; Popa, A.M.; Miclean, M.; Hoaghia, M.A.; Cadar, O.; Carstea, E.M. Spatial variation of organochlorine pesticides and dissolved organic matter in urban closed lakes. *J. Environ. Sci. Health B* **2020**, *55*, 329–341. [CrossRef]
8. Miclean, M.; Cadar, O.; Levei, E.A.; Roman, R.; Ozunu, A.; Levei, L. Metal (Pb, Cu, Cd, and Zn) transfer along food chain and health risk assessment through raw milk consumption from free–range cows. *Int. J. Environ. Res. Public Health* **2019**, *16*, 4064. [CrossRef]
9. United Nations Environment Programme. UNEP 2009 Annual Report. Available online: http://hdl.handle.net/20.500.11822/7824 (accessed on 22 November 2023).
10. UNEP. 'Chemicals Proposed for Listing under the Convention', Stockholm Convention. 2018. Available online: http://chm.pops.int/TheConvention/ThePOPs/ChemicalsProposedforListing/tabid/2510/Default.aspx (accessed on 22 November 2023).
11. Tzanetou, E.N.; Karasali, H. A Comprehensive review of organochlorine pesticide monitoring in agricultural soils: The silent threat of a conventional agricultural past. *Agriculture* **2022**, *12*, 728. [CrossRef]
12. Esposito, M.; Canzanella, S.; Iaccarino, D.; Pepe, A.; Di Nocera, F.; Bruno, T.; Marigliano, L.; Sansone, D.; Hochscheid, S.; Gallo, P.; et al. Trace Elements and Persistent Organic Pollutants in Unhatched Loggerhead Turtle Eggs from an Emerging Nesting Site along the Southwestern Coasts of Italy, Western Mediterranean Sea. *Animals* **2023**, *13*, 1075. [CrossRef]
13. Mercanti, T.; El Hachmi, M.; Falcinelli, S.; Sebastiani, B. occurrence of persistent organochlorine pollutants in sediments from Lake Piediluco, Italy. *Environments* **2023**, *10*, 120. [CrossRef]
14. Li, Z. Prioritizing agricultural pesticides to protect human health: A multi–level strategy combining life cycle impact and risk assessments. *Ecotox. Environ. Saf.* **2022**, *242*, 113869. [CrossRef] [PubMed]
15. Kalyabina, V.; Esimbekova, E.N.; Kopylova, K.V.; Kratasyuk, V. Pesticides: Formulants, distribution pathways and effects on human health—A review. *Toxicol. Rep.* **2021**, *8*, 1179–1192. [CrossRef] [PubMed]
16. Li, Z.; Fantke, P. Framework for defining pesticide maximum residue levels in feed: Applications to cattle and sheep. *Pest. Manag. Sci.* **2023**, *79*, 748–759. [CrossRef] [PubMed]
17. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on Undesirable Substances in Animal Feed. *Off. J. Eur. Union L* **2002**, *140*, 10–22. Available online: https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32002L0032 (accessed on 15 September 2023).

18. Li, Z.; Xiong, J.; Fantke, P. Screening of pesticide distributions in foods of animal origin: A matrix–based approach for biotransfer factor modeling of grazing mammals. *Environ. Sci. Process. Impacts* **2022**, *24*, 609–624. [CrossRef]
19. Rychen, G.; Jurjanz, S.; Fournier, A.; Toussaint, H.; Feidt, C. Exposure of ruminants to persistent organic pollutants and potential of decontamination. *Environ. Sci. Pollut. Res. Int.* **2014**, *21*, 6440–6447. [CrossRef]
20. Feidt, C.; Ounnas, F.; Julien–David, D.; Jurjanz, S.; Toussaint, H.; Jondreville, C.; Rychen, G. Relative bioavailability of soil–bound polychlorinated biphenyls in lactating goats. *J. Dairy Sci.* **2013**, *96*, 3916–3923. [CrossRef]
21. Jia, Q.; Liao, G.; Chen, L.; Qian, Y.; Yan, X.; Qiu, J. Pesticide residues in animal-derived food: Current state and perspectives. *Chemosphere* **2024**, *438*, 137974. [CrossRef]
22. Stiefel, C.; Stintzing, F. Endocrine–active and endocrine–disrupting compounds in food—Occurrence, formation and relevance. *NFS J.* **2023**, *31*, 57–92. [CrossRef]
23. Ramezani, S.; Mahdavi, V.; Gordan, H.; Rezadoost, H.; Oliver Conti, G.; Mousavi Khaneghah, A. Determination of multi–class pesticides residues of cow and human milk samples from Iran using UHPLC–MS/MS and GC–ECD: A probabilistic health risk assessment. *Environ. Res.* **2022**, *208*, 112730. [CrossRef]
24. Mamontova, E.A.; Tarasova, E.N.; Mamontov, A.A.; Kuzmin, M.I.; McLachlan, M.S.; Khomutova, M.I. The influence of soil contamination on the concentrations of PCBs in milk in Siberia. *Chemosphere* **2007**, *67*, S71–S78. [CrossRef] [PubMed]
25. Tremolada, P.; Guazzoni, N.; Parolini, M.; Rossaro, B.; Bignazzi, M.M.; Binelli, A. Predicting PCB concentrations in cow milk: Validation of a fugacity model in high–mountain pasture conditions. *Sci. Total Environ.* **2014**, *487*, 471–480. [CrossRef] [PubMed]
26. EU, EIP Brochure. European Union Brochure: Sustainable Livestock Farming. Innovation for farm Resilience and Profitability. Available online: https://ec.europa.eu/eip/agriculture/sites/default/files/eip-agri_brochure_sustainable_livestock_2019_en_web.pdf (accessed on 27 November 2023).
27. *ISO 6468:1996*; Water Quality. Determination of Certain Organochlorine Insecticides, Polychlorinated Biphenyls and Chlorobenzenes. Gas Chromatographic Method after Liquid–liquid Extraction. ISO: Geneva, Switzerland, 1996.
28. Popa, C.L.; Dontu, S.I.; Carstea, E.M.; Levei, E.A.; Ioja, C.; Popa, A.M.; Miclean, M.; Cadar, O. Organochlorine pesticides and dissolved organic matter within a system of urban exorheic lakes. *Environ. Monit. Assess.* **2019**, *192*, 59. [CrossRef] [PubMed]
29. US Environmental Protection Agency. *Ultrasonic Extraction, Test Methods for Evaluating Solid Waste*; Method 3550C; US Environmental Protection Agency: Washington, DC, USA, 2000.
30. De Vries, W.; Römkens, P.F.A.M.; Schütze, G. Critical soil concentrations of cadmium, lead, and mercury in view of health effects on humans and animals. *Rev. Environ Contam. Toxicol.* **2007**, *191*, 91–130. [PubMed]
31. Rodrigues, S.M.; Pereira, M.E.; Duarte, A.C.; Römkens, P.F.A.M. Soil–plant–animal transfer models to improve soil protection guidelines: A case study from Portugal. *Environ. Int.* **2012**, *39*, 27–37. [CrossRef] [PubMed]
32. Le Faouder, J.; Bichon, E.; Brunschwig, P.; Landelle, R.; Andre, F.; Le Bizec, B. Transfer assessment of fipronil residues from feed to cow milk. *Talanta* **2007**, *73*, 710–717. [CrossRef]
33. Order 756. Order of the Ministry of Water, Forestry and Environmental Protection for the Approval of the Regulation Regarding the Assessment of Environmental Pollution, No. 756 of 3 November 1997. Published in the Official Gazette of Romania, No. 303 Bis of 6 November 1997. 1997. Available online: https://legislatie.just.ro/Public/DetaliiDocumentAfis/13572 (accessed on 10 September 2023). (In Romanian).
34. Wang, X.; Wang, D.; Qin, X.; Xu, X. Residues of organochlorine pesticides in surface soils from college school yards in Beijing, China. *J. Environ. Sci.* **2008**, *20*, 1090–1096. [CrossRef]
35. Westbom, R.; Hussen, A.; Megersa, N.; Retta, N.; Mathiasson, L.; Björklund, E. Assessment of organochlorine pesticide pollution in Upper Awash Ethiopian state farm soils using selective pressurised liquid extraction. *Chemosphere* **2008**, *72*, 1181–1187. [CrossRef]
36. Yu, H.Y.; Li, F.B.; Yu, W.M.; Li, Y.T.; Yang, G.Y.; Zhou, S.G.; Zhang, T.B.; Gao, Y.X.; Wan, H.F. Assessment of organochlorine pesticide contamination in relation to soil properties in the Pearl River Delta, China. *Sci. Total Environ.* **2013**, *447*, 160–168. [CrossRef]
37. Zhou, Q.; Wang, J.; Meng, B.; Cheng, J.; Lin, G.; Chen, J.; Zheng, D.; Yu, Y. Distribution and sources of organochlorine pesticides in agricultural soils from central China. *Ecotox. Environ. Saf.* **2013**, *93*, 163–170. [CrossRef]
38. Gonzalez, M.; Miglioranza, S.B.; Aizpun, J.E.; Isla, F.I.; Pena, A. Assessing pesticide leaching and desorption in soils with different agricultural activities from Argentina (Pampa and Patagonia). *Chemosphere* **2010**, *81*, 351–358. [CrossRef] [PubMed]
39. Barron, M.G.; Ashurova, Z.J.; Kukaniev, M.A.; Avloev, H.K.; Khaidarov, K.K.; Jamshedov, J.N.; Rahmatullova, O.S.; Atolikshoeva, S.S.; Mamadshova, S.S.; Manzenyuk, O. Residues of organochlorine pesticides in surface soil and raw foods from rural areas of the Republic of Tajikistan. *Environ. Pollut.* **2017**, *224*, 494–502. [CrossRef] [PubMed]
40. Fosu-Mensah, B.Y.; Okoffo, E.D.; Darko, G.; Gordon, C. Assessment of organochlorine pesticide residues in soils and drinking water sources from cocoa farms in Ghana. *Springerplus* **2016**, *24*, 869. [CrossRef] [PubMed]
41. Zhong, G.; Tang, J.; Zhao, Z.; Pan, X.; Chen, Y.; Li, J.; Zhang, G. Organochlorine pesticides in sediments of Laizhou Bay and its adjacent rivers, North China. *Mar. Pollut. Bull.* **2011**, *62*, 2543–2547. [CrossRef] [PubMed]
42. Order 396. Order of the Minister of Agriculture, Food and Forests, the Minister of Health and Family and the Minister of Water and Environmental Protection No. 396 of 2 September 2002 Regarding the Prohibition of the Use on Romanian Territory of Phytosanitary Products Containing Certain Active Substances. Published in the Official Gazette of Romania, No. 829 of 18 November 2002. 2002. Available online: https://legislatie.just.ro/Public/DetaliiDocumentAfis/39933 (accessed on 15 September 2023). (In Romanian).

43. Ordinance 7. The Government of Romania. Ordinance no. 7 of 18 January 2023 Regarding the Quality of Water Intended for Human Consumption. Published in the Official Gazette of Romania, No. 63 of 25 January 2023. Available online: https://legislatie.just.ro/Public/DetaliiDocument/264337 (accessed on 25 September 2023). (In Romanian).
44. Polanco Rodríguez, A.G.; Araujo León, J.A.; Cetz, R.L.; Long, D.; Álvarez Cervera, F.J.; Barache, U.; Rosas Sánchez, D.H. Organochlorine pesticides in the drinking water of Merida and its Metropolitan Zone, a Karst Region. *Urban Water J.* **2022**, *19*, 40–50. [CrossRef]
45. Guo, J.; Chen, W.; Wu, M.; Qu, C.; Sun, H.; Guo, J. Distribution, sources, and risk assessment of organochlorine pesticides in water from Beiluo River, Loess Plateau, China. *Toxics* **2023**, *11*, 496. [CrossRef] [PubMed]
46. Jin, X.; Liu, Y.; Qiao, X.; Guo, R.; Liu, C.; Wang, X.; Zhao, X. Risk assessment of organochlorine pesticides in drinking water source of the Yangtze River. *Ecotoxicol. Environ. Saf.* **2019**, *182*, 109390. [CrossRef]
47. Costera, A.; Feidt, C.; Marchand, P.; Le Bizec, B.; Rychen, G. PCDD/F and PCB transfer to milk in goats exposed to a long–term intake of contaminated hay. *Chemosphere* **2006**, *64*, 650–657. [CrossRef]
48. Mantovani, A.; Frazzoli, C. Risk assessment of toxic contaminants in animal feed. *CAB Rev. Perspect.* **2010**, *5*, 1–14. [CrossRef]
49. Panseri, S.; Biondi, P.A.; Vigo, D.; Communod, R.; Chiesa, L.M. Occurrence of organochlorine pesticides residues in animal feed and fatty bovine tissue. *Food Ind.* **2013**, *13*, 261–283. [CrossRef]
50. Deka, S.C.; Barman, N.; Baruah, A.A.L.H. Monitoring of pesticide residues in feed, fodder and butter in Assam. *Pestic. Res. J.* **2004**, *16*, 86–89.
51. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on Maximum Residue Levels of Pesticides in or on Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC. *Off. J. Eur. Union L* **2005**, *70*, 1–16. Available online: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex:32005R0396 (accessed on 30 September 2023).
52. Order 23. Order of the President of the National Sanitary Veterinary and Food Safety Authority approving the Sanitary Veterinary and Food Safety Normative on the Establishment of the Maximum Admitted Residue Levels of Pesticides in Content or on Foodstuffs of Animal Origin, No. 23/26.12.2007. Published in the Official Gazette of Romania, No. 138 of 26 December 2007. 2007. Available online: https://www.cdep.ro/pls/legis/legis_pck.htp_act?ida=70288 (accessed on 2 September 2023). (In Romanian).
53. Gebremichael, S.; Birhanu, T.; Tessema, D.A. Analysis of organochlorine pesticide residues in human and cow's milk in the towns of Asendabo, Serbo and Jimma in South–Western Ethiopia. *Chemosphere* **2013**, *90*, 1652–1657. [CrossRef] [PubMed]
54. Soares, V.A.; Kus, M.M.; Peixoto, A.L.C.; Carrocci, J.S.; Salazar, R.F.S.; Izário–Filho, H.J. Determination of nutritional and toxic elements in pasteurized bovine milk from Vale do Paraiba region (Brazil). *Food Control* **2010**, *21*, 45–49. [CrossRef]
55. EU Pesticide Database. Search Active Substances. 2022. Available online: https://ec.europa.eu/food/plants/pesticides/eu-pesticides-database_en (accessed on 28 December 2023).
56. Kampire, E.; Kiremire, B.T.; Nyanzi, S.A.; Kishimba, M. Organochlorine pesticide in fresh and pasteurized cow's milk from Kampala markets. *Chemosphere* **2011**, *84*, 923–927. [CrossRef] [PubMed]
57. Deti, H.; Hymete, A.; Bekhit, A.A.; Mohamed, A.M.I.M.; Bekhit, A.E.-D.A. Persistent organochlorine pesticides residues in cow and goat milks collected from different regions of Ethiopia. *Chemosphere* **2014**, *106*, 70–74. [CrossRef] [PubMed]
58. Isaq, Z.; Nawaz, M.A. Analysis of contaminated milk with organochlorine pesticide residues using gas chromatography. *Int. J. Food Prop.* **2018**, *21*, 879–891. [CrossRef]
59. Arif, A.M.; Javed, I.; Ayaz, M.; Abdullah, M.; Imran, M.; Shahbaz, M.; Gondal, T.A.; Ali, M.; Iqbal, Z.; Iqbal, Z.; et al. Organochlorine pesticide residues in raw milk samples collected from dairy farms and urban areas of Lahore district, Pakistan. *J. Food Sci. Technol.* **2021**, *58*, 129–137. [CrossRef]
60. Hasan, G.M.M.A.; Das, A.K.; Satter, M.A. Multi residue analysis of organochlorine pesticides in fish, milk, egg and their feed by GC-MS/MS and their impact assessment on consumers health in Bangladesh. *NFS J.* **2022**, *27*, 28–35. [CrossRef]
61. Rosenbaum, R.K.; McKone, T.E.; Jolliet, O. CKow—A More Transparent and Reliable Model for Chemical Transfer to Meat and Milk, March 2009. Available online: https://www.osti.gov/servlets/purl/971640 (accessed on 8 November 2023).
62. The Environment Agency. Science Report—SC030197/SR4. Verification of Bioaccumulation Models for Use in Environmental Standards. Part C: Human Food Chain Models. p. 5. Available online: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/291724/scho0507bmpr%E2%80%93e%E2%80%93e.pdf (accessed on 8 November 2023).
63. Huuskonen, A.; Tuomisto, L.; Kauppinen, R. Effect of drinking water temperature on water intake and performance of dairy calves. *J. Dairy Sci.* **2011**, *94*, 2475–2480. [CrossRef]
64. Travis, C.C.; Arms, A.D. Bioconcentration of organics in beef, milk, and vegetation. *Environ. Sci. Technol.* **1988**, *22*, 271–274. [CrossRef]
65. Leeman, W.R.; Van Den Berg, K.J.; Houben, G.F. Transfer of chemicals from feed to animal products: The use of transfer factors in risk assessment. *Food Addit. Contam. A* **2007**, *24*, 1–13. [CrossRef] [PubMed]

66. Amutova, F.; Delannoy, M.; Baubekova, A.; Konuspayeva, G.; Jurjanz, S. Transfer of persistent organic pollutants in food of animal origin—Meta-analysis of published data. *Chemosphere* **2021**, *262*, 128531. [CrossRef] [PubMed]
67. EFSA (European Food Safety Authority); Ardizzone, M.; Binaglia, M.; Cottrill, B.; Cugier, J.-P.; Ferreira, L.; Gomez Ruiz, J.A.; Innocenti, M.; Ioannidou, S.L.; Lopez Puente, S.; et al. Scientific report on the animal dietary exposure: Overview of current approaches used at EFSA. *EFSA J.* **2019**, *17*, 5896.