Study Of The Effect Of Accumulated Cypermethrin Residues In Cattle Meat In Some Areas Of Karbala Province

Zainab Ali Jabur[1], Assist. Prof. Dr. Kadhim Saleh Kadhim[2], Assist. Prof. Dr. Ali Hussein Fadhil[3]

KEYWORDS
Cattle Meat, Pesticides, Residues, Cypermethrin, HPLC

ABSTRACT
The synthetic pesticide cypermethrin is used in livestock and agriculture in Iraq. Due to the excessive use of these pesticides, they accumulate in the first feed of animals, causing health problems. Accordingly, the following study was designed for demonstration determination of synthetic cypermethrin by HPLC (High Performance Liquid Chromatography) in cattle meat samples. One hundred samples were collected from various local markets and butcher shops from (Al- Hassainya, City Center, Al-Hur, Twairij, and Ain Al-Tamer) Kerbala-Iraq. Five grams were collected separately from each sample. Then, the samples (cattle meat) were crushed and homogenized, after which 25 ml of acetonitrile was added for extraction the pesticides residues from these sample then the reagents were added to extract then the extract were injected to HPLC instrument. The obtained results revealed that (Al-Hassainya, City Center, Al-Hur, Twairij, and Ain Al-Tamer) (0.1569; 5.408; 6.409; 5.509; 5.408ppm), in cattle meat, respectively. From the results in this study, Al-Hur cattle meat (6.409±0.1137) was contaminated more than all regains of Karbala distract. This study showed the dangers of the presence of synthetic cypermethrin in cattle meat, threatening consumer health.

1. Introduction

Cypermethrin (CYP) is a man-made toxic chemical compound that belongs to the environmentally polluting pyrethroid insecticides[1].Cypermethrin is a synthetic pyrethroid derived from pyrethrin, produced by Chrysanthemum spp. a naturally occurring substance in the flower head. It can be used in many areas such as family, agriculture and livestock control against cockroaches, mosquitoes, lice, ticks and spiders[2].Since the late 1970s, cypermethrin has been utilized extensively around the world for almost 40 years. a[3,4]. About 80% of the pesticides imported into Iraq each year are insecticides. [5].

Most of the CYPs produced worldwide are used to inject or spray insecticides (such as ticks, ants and scabies) [6]. It is also used as a home remedy and remains in the air and on the surface. For three months, walls and furniture. It is the fourth leading source of pesticide poisoning among California pest control professionals[7,8].

Pyrethroids are pyrethrin insecticides that combine potency, safety, low environmental impact and safety. Excessive use of pyrethroids in plant protection products and livestock can lead to the spread of parasites in animal tissue, milk, eggsand honey. Veterinary treatments include earplugs, drops, sprays and dips.

Due to their animal metabolism, they tend to bioaccumulate, becoming a potential source of contamination through foods. As a result of their physicochemical properties and toxicity, pyrethroids in foods of animal origin must be monitored, control the quality of food and thereby prevent risks to human health[9].

The nervous system is a target organ of CYP. It directly affects the sodium channel, CYP slows the sodium channel closure for a few seconds. [10,11].

Crimea-Congo hemorrhagic disease (CCHF) causes hemorrhagic disease with a mortality rate as high as percent in humans and is not usually seen in animals[12].

Crimean-Congo Hemorrhagic Virus (CCHFV) in different Iraqi provinces. In the 2022 distripution, there were 212 human cases of CCHFV between January 1 and May 22. Among these 97 (46%) confirmed cases by molecular photocopying (RT-PCR) and 115 (54%) were suspicious. Many animal-borne diseases have been reported. Almost half (48%) of proved incident were reported in

Dhi Qar district, while others were reported in Missan, Muthanna, Wasit, Diwaniya, Karkh/Baghdad, Rusafa/Baghdad, Kirkuk, Basra, Najaf, Nineveh and Babylonian states. Karbala. Contributing factors to the resurgence of CCHFV include the lack of veterinary services at the time of the COVID-19 pandemic and the unauthorized killing of animals outdoor of slaughterhouses. This result to a major drought that occurred as viral vectors[13]. Crimean-Congo hemorrhagic fever (CCHF) is spread by ticks and is classified as a viral infection. CCHF is a fatal disease in Iraq and has been reported sporadically since the first report in 1979. The last epidemic and its fatal consequences in 2021-2023 are important for this research. CCHF is a tick-borne disease that poses a major public health, social and economic problem. The geographical distribution of CCHF is closely related to the distribution of Hyalomma ticks. Therefore, predicting and mapping the spread of disease-associated environmental conditions provides useful information for establishing early warning systems based on preventive measures to reduce the spread of CCHF risk[14].controlled substances are chemicals with different properties, effects and toxicity. Considering their positive effects and toxicological properties, synthetic pyrethroids containing have entered practical applications[15]. Intensive CIS-cypermethrin treatment reduced the infection rate by % (almost 100%) after one day of treatment.

Many methods have been published for the determination of pyrethroid residues in different species using different methods of separation and detection, but very few principles have been published for the analyses of pyrethroids residues in cattle meat.

Most of the analysis methods are depends on the extraction of the material including an organic solvent (petroleum ether, acetone, n-hexane), partial cleaning of the solvent and / or adsorption chromatography, and determine the remainder. [23].

The object of this study was to disclosure and validation of an analytical technique for determining residues of the ectoparasiticide cypermethrin in beef and comparing maximum residue limits with residues in foodstuffs.

2. Methodology

Chemicals and Reagents:

Cypermethrin, methanol HPLC grade, Deionized water, Acetonitrile, n-methylimidazole, trichloroacetic acid AR.

Samples collection:

One hundred sample of fresh cattle meat 50gm for each sample in polyethylene bags were collected randomly from local market from five district in Kerbala province (20 samples from Al-Hassainya, 20 samples from City Center, 20 samples from Al-Hur, 20 samples from Twairij, and20 samples from Ain Al-Tamer) samples were obtained as sold the public and transferred as soon as possible in an ice box 4±1ºC to laboratory with a minimum of delay.

Method:

Preparation of reagents:

Reagent(a) Acetonitrile with n-methylimidazole is widely used as a solvent in various chemical reactions, especially in organic synthesis. So, the solvent is prepared by mixing 5ml of n-methylimidazole (NMI) with 5ml of acetonitrile (ACN) [24]

Reagent(b) Acetonitrile (ACN) with Trichloroacetic acid (TCA) is widely used as a solvent in various chemical reactions, especially in organic synthesis. So, the solvent is prepared by mixing 10ml of 0.1 % Trichloroacetic acid (TCA) with 5ml of acetonitrile (ACN) [24]

Extraction:

All samples were performed in the Nutrition and Public Health Laboratory at the College of Veterinary Medicine/ Karbala University. Samples were taken from fresh cattle meat from local Iraqi

market in Kerbela province from different district (Al-Hassainya, Al-Hur, Ain Al-Tamur, Center of Kerbela and Twuriji).

The extraction method used in this study is a modification of the multiple residue analysis method, as described[25].

In this study, 5 grams were collected separately from each sample. Then, the samples (beef) were crushed and homogenized, after which 25 ml of acetonitrile was added., then the sample was shaken with a magnetic stirrer for 30 minutes, after which the sample was left to stand for 15 minutes, then the liquid was removed Extraction is applied to remove solids at 45,000 rpm for 15 minutes, the extraction was repeated on the same sample with the same steps as the previous steps. After collecting the liquid from the centrifuge, it is placed in an Erlenmeyer flask and placed in a fume hood in a water bath with a temperature below 50 ° C until the liquid is completely dry. After that, the sediment remaining after drying the flask is washed with 1 ml of acetonitrile, then collected in an opaque glass vial and 100 µm of solvent is added. A, then 150 µm solvent B [26]

The samples were sent to the laboratory of the Materials Research Department at the Ministry of Science and Technology in Baghdad for the purpose of examining the samples using a high-performance liquid chromatography device, and then the samples were filtered using a special filter with a size of 25mm to be Ready to read with (HPLC High-Performance Liquid chromatography – Japan [27]

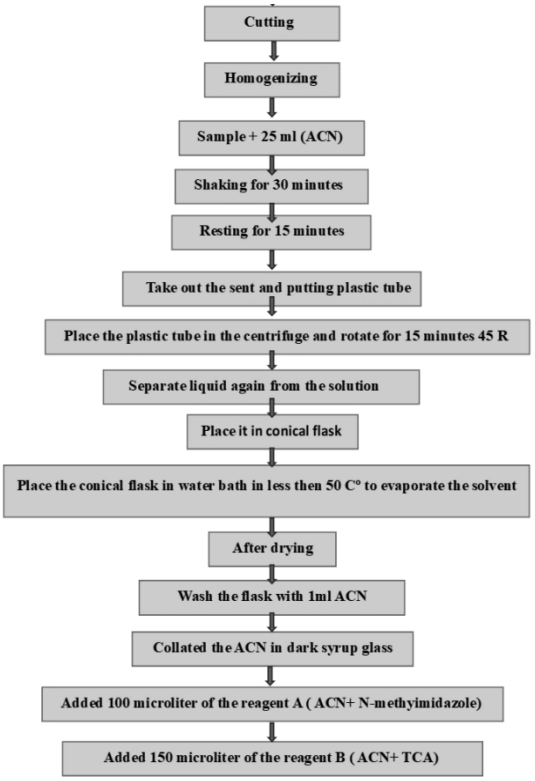

Figure (1): Steps for extracting cypermethrin from cattle meat sample

P

S anol to get a concentration 100 ppm (100 ml/L). Then diluted to (10,5,2.5,3.3…. etc.) ppm. Calibration solutions were prepared in the Laboratory of the Materials Department of the Iraqi Ministry of Science and Technology in Baghdad.

Statistical Method:

The Pad graph Prism program was used to create graphs, find the average and standard deviation, and compare them with the ratios approved by the World Health Organization (WHO) for the year 2023 for cypermethrin and the year 2022 for ivermectin. One Way ANOVA and t. Test were used:

3. Results and discussion

Table (1): cypermethrin concentrations in cattle meat samples PPM(Means±SD).

Group	WHO	Al-Hassainya	Center	Twairij	Al-Hur	Ain Al-Tumar
Result	0.03	0.1569 ±0.0018	5.408 ±0.0682	5.509 ±0.0554	6.409 ±0.113	5.408 ±0.1038

The current study showed that all meat samples collected from different district of Kerbala province (Al-Hassainya, City Center, Al-Hur, Twairij, and Ain Al-Tamer) (0.1569±+0.0018; 5.408±0.0682; 6.409±0.1137; 5.509±0.0554; 5.408±0.1038 ppm) respectively had a highly significant difference compared with maximum residue level of cypermethrin pesticide in the world health organization (WHO) (0.03) index for 2023 year. ————On the other hand, it was found that Al-Hur district 6.409±0.1137 was

highly significant from (Al-Hassainya, center of Kerbala, Twairij and Ain Al-Tumar) as shown in figure (1) and table (1).

Table (2) level of cypermethrin residues (ppm), and violation in cattle meat samples collected from different region of Karbala

Seq.	Sample region	No.of total sample	No. of +ve sample	No. of-ve sample	MRLS	Means±SD	%Violation
1	Al-Hassainya	20	10	10	0.03	0.1569±0.0018	500
2	Center	20	12	8	0.03	5.408±0.0682	1800
3	Twairij	20	15	5	0.03	5.509±0.0554	1800
4	Al-Hur	20	17	3	0.03	6.409±0.1137	21000
5	Ain Al-Tumar	20	16	4	0.03	5.408±0.1038	1800
Sum	5	100	70	30			

Discussion:

The current study showed that all meat samples collected from different district of Kerbala province (Al-Hassainya, City Center, Al-Hur, Twairij, and Ain Al-Tamer) (0.1569+0.0018; 5.408±0.0682; 6.409±0.1137; 5.509±0.0554; 5.408±0.1038 ppm had a highly significant difference compared with maximum residue level of cypermethrin pesticide in the world health organization (WHO) (0.03) index for 2023 year. The other researchers find that in the muscles the residue was detected only in day 3 samples, the balance is 24 µg / kg. The highest level of cypermethrin (2.75 µg L-1, 14.7 µg kg-1 ww) was found in beef, and a close relationship between pesticide levels and fat in beef.[6,28]and this findings were strongly agree with the result of the presented study .

While the other researcher finds that, The ARfD of the maximal levels of compounds in milk (CHL, 45.7 µg L-1) and cattle meat (CYP, 14.7 µg kg-1 ww) was estimated. The acute reference level (ARfD) is 26 ng kg-1 body weight-1. day and 21ng kg-1 body weight-1CYP. If these values are lower than those recommended by Database Properties (PPDB 2017) 200 µg kg-1bw day-1 for CYP [29] and these result also agree with the result of presented study.

moreover Z-cypermethrin was compared in only one sample (0.015 mg kg-1 after 2 days). Z-cypermethrin was detected in almost half of the samples (above limits of detection (LOD), 0.002 mg kg-1) but below the limit of quantification (LOQ) (0.01 mg kg-1). In the Jersey study, a residue of 0.025 mg was observed 1 day after treatment. Counts were lost in three out of five treated milks one day after administration and in two out of five on days 2 and 3. Around half of the remaining Jersey groups had the product is detectable but under the LOQ.[30] and this result was on side with presented research.

In contrast to the results of this study, researchers found Cypermethrin was not present in animal samples in winter, while in summer, animal samples were 0.066 ± 0.046, 0.0002 ± 0.0001, 0.0003 ± 0.0002 and 0.0004 ± 0.00. In the fat sample, cypermethrin was detected with an average value of 0.159 ± 0.110 and 39.872 ± 28.028, respectively[31].

Reference

[1] Aioub AAA, Zuo Y, Li Y, et al. Transcriptome analysis of Plantago major as a phytoremediator to identify some genes related to cypermethrin detoxification. Environ Sci Pollut Res. 2021;28:5101-5115.

[2] Shilpakar O, Karki B. Cypermethrin poisoning manifesting with prolonged bradycardia: A case report. Toxicol reports. 2021;8:10-12.

[3] Yousef MI, Ibrahim HZ, Yacout MHM, Hassan AA. Effects of cypermethrin and dimethoate, on some physiological and biochemical parameters in Barki sheep. Published online 1998.

[4] SCOTTISHSTATUTORYINST R. The Pesticides (Maximum Residue Levels in Crops, Food and Feeding Stuffs)(Scotland) Amendment (No. 2) Regulations 2002. Published online 2003.

[5] Al-Autaish HHN. Clinical study on toxicity of cypermethrin in Arrabi sheep. Published online 2010.

[6] Jabbar MK. Effect of Toxicity Cypermethrin in Animals: A Review. Int J Sci Trends. 2022;1(2):1-14.

[7] cox. No Title. 1996;16(2):15-20.

[8] Thompson M. DOMESTIC OR COMMERCIAL PESTICIDE USE: COULD YOU KILL OR BE KILLED? AN ISSUE THAT AFFECTS EVERYONE, ALL THE TIME!

[9] Dallegrave A, Pizzolato TM, Barreto F, Eljarrat E, Barceló D. Methodology for trace analysis of 17 pyrethroids and chlorpyrifos in foodstuff by gas chromatography–tandem mass spectrometry. Anal Bioanal Chem. 2016;408:7689-7697.

[10] FAO/WHO Meeting JFEC on FA, Organization WH. Compendium of Food Additive Specifications: Addendum 8. Vol 52. Food & Agriculture Org.; 2000.

[11] Seth A, Song KP, Pettit R. Synergy, managerialism or hubris? An empirical examination of motives for foreign acquisitions of US firms. J Int Bus Stud. 2000;31:387-405.

[12] Altaliby MAS, Esmaeel SA, Hussain KJ. Seroprevalence of Crimean-Congo Haemorrhagic Fever in sheep and goats in Iraq. Bulg J Vet Med. 2021;26:1-6.

[13] Al Salihi KA, Younise H, Zuhair Mahmoud Z, Hussain T. The 2022 crimean-congo hemorrhagic fever outbreak in Iraq. Austral J Vet Sci. 2024;56(1):35-40.

[14] Khwarahm NR. Predicting the spatial distribution of Hyalomma ssp., vector ticks of Crimean–Congo Haemorrhagic fever in Iraq. Sustainability. 2023;15(18):13669.

[15] Buczek A, Bartosik K, Kuczyński P. The toxic effect of permethrin and cypermethrin on engorged Ixodes ricinus females. 2014;21(2):259-262. doi:10.5604/1232-1966.1108587

[16] Akre CJ, MacNeil JD. Determination of eight synthetic pyrethroids in bovine fat by gas chromatography with electron capture detection. J AOAC Int. 2006;89(5):1425-1431.

[17] Barbini DA, Vanni F, Girolimetti S, Dommarco R. Development of an analytical method for the determination of the residues of four pyrethroids in meat by GC–ECD and confirmation by GC–MS. Anal Bioanal Chem. 2007;389:1791-1798.

[18] Cheng J, Liu M, Yu Y, et al. Determination of pyrethroids in porcine tissues by matrix solid-phase dispersion extraction and high-performance liquid chromatography. Meat Sci. 2009;82(4):407-412.

[19] 19. Goulart SM, de Queiroz MELR, Neves AA, de Queiroz JH. Low-temperature clean-up method for the determination of pyrethroids in milk using gas chromatography with electron capture detection. Talanta. 2008;75(5):1320-1323.

[20] Cencič-Kodba Z, Brodnjak-Vončina D. A Rapid Method for the Determination of Organochlorine, Pyrethroid

Pesticides and Polychlorobiphenyls in Fatty Foods Using GC with Electron Capture Detection.; 2007.

[21] Kuang H, Miao H, Hou XL, et al. Determination of pyrethroid residues in pork muscle by immunoaffinity cleanup and GC-ECD. Chromatographia. 2009;70:995-999.

[22] Stefanelli P, Santilio A, Cataldi L, Dommarco R. Multiresidue analysis of organochlorine and pyrethroid pesticides in ground beef meat by gas chromatography-mass spectrometry. J Environ Sci Heal Part B. 2009;44(4):350-356.

[23] Poole CF. Selectivity evaluation of extraction systems. J Chromatogr A. Published online 2023:463939.

[24] Zhao D, Liu M, Zhang J, Li J, Ren P. Synthesis, characterization, and properties of imidazole dicationic ionic liquids and their application in esterification. Chem Eng J. 2013;221:99-104.

[25] Lentza-Rizos C, Avramides EJ, Visi E. Determination of residues of endosulfan and five pyrethroid insecticides in virgin olive oil using gas chromatography with electron-capture detection. J Chromatogr A. 2001;921(2):297-304.

[26] Zainudin BH, Abu Bakar NK, Muhamad H. Determination of cypermethrin in palm oil matrices. Eur J Lipid Sci Technol. 2009;111(10):1014-1019. doi:10.1002/ejlt.200800240

[27] Almashhadany DA. Monitoring of antibiotic residues among sheep meat in Erbil city and thermal processing effect on their remnants. Iraqi J Vet Sci. 2020;34(2):217-222.

[28] Sarmiento-Santos J, Souza MNB, de Souza D, Vanin FM. Evaluation of Brazilian pesticide residue reported by monitoring programs considering foods offered to childrens. Food Control. 2024;163:110483.

[29] Jardim ANO, Brito AP, van Donkersgoed G, Boon PE, Caldas ED. Dietary cumulative acute risk assessment of organophosphorus, carbamates and pyrethroids insecticides for the Brazilian population. Food Chem Toxicol. 2018;112:108-117.

[30] 30. Rothwell JT, Burnett TJ, Hacket K, Chevis R, Lowe LB. Residues of zeta-cypermethrin in bovine tissues and milk following pour-on and spray application. Pest Manag Sci Former Pestic Sci. 2001;57(11):993-999.

[31] 31. FA K, N S AA, I Ali O. Pesticides residues in retail meat and offal. J Vet Med Res. 2019;26(1):101-114.