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Assessment of commonly used insecticide residues using gas chromatography in buffalo liver samples collected from North India

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ABSTRACT: Gas Chromatography was employed to quantify the residue levels of frequently used insecticides in randomly collected buffalo liver samples from North India. A total of 100 samples were screened for organophosphate pesticides, while 50 samples each were analysed for organochlorine and synthetic pyrethroid compounds. Samples were collected between December, 2014 to March, 2017 from Haryana and Delhi. Liver samples were treated with the procedure outlined by Dutch ministry of public health, welfare and sports (AMPRF, 1996). Nine organophosphate insecticides were investigated, namely dichlorovos, chlorpyrifos methyl, fenitrothion, pirimiphos methyl, malathion, Chlorpyriphos, quinalphos, ethion, and edifenphos. In addition, eight organochlorine insecticides (α-HCH, β-HCH, γ-HCH, δ-HCH, aldrin, dicofol, α-endosulfan, β-endosulfan) and five synthetic pyrethroid insecticides (λ-cyhalothrin, cypermethrin, fenvelrate, T-fluvalinate, and deltamethrin) were included in the analysis. The estimated total pesticide burden was 5.75 ppb for organophosphate residues and 53.89 ppb for the combined organochlorine and synthetic pyrethroid residues. γ-HCH concentrations exceeded the Maximum Residue Limit (MRL) as defined by the Codex Alimentarius Commission (2006) in 23 of the samples analysed. β-endosulfan recorded the highest mean residue concentration among all compounds detected in buffalo liver.

Keywords: Buffalo Liver, Gas Chromatography, Organochlorines, Organophosphates, Synthetic pyrethroids

India has transformed from a food-deficit nation in the early 1950s to a major producer and exporter of food grains. Foodgrain production increased from approximately 50.8 million tonnes in 1950–51 to a record 357.3 million tonnes in 2024–25, largely due to the Green Revolution and the adoption of improved crop varieties, expanded irrigation infrastructure, fertilizers, and pesticides (Eliazer Nelson *et al*., 2019; Srivastava *et al*., 2025). India is among the world's leading producers of agrochemicals and has emerged as a major exporter of crop protection products. The growth of the Indian agrochemical sector has been driven by increasing agricultural intensification, rising food demand, expansion of domestic manufacturing capacity, and growing participation in global agrochemical supply chains (Chakraborty and Ebihara, 2025; Janaki Rani *et al*., 2025). Pesticides provide significant agricultural and public health benefits when applied according to recommended guidelines, targeting specific pests at appropriate doses and frequencies. However, indiscriminate or excessive use can result in environmental contamination and adverse human health effects. Persistent pesticide residues may accumulate in environmental compartments and biomagnify through food webs, leading to contamination of food products of animal origin, including meat, milk, and eggs, thereby posing potential risks to consumers (Beyuo *et al*., 2024; Schopf *et al*., 2023; Shekhar *et al*., 2024). As per the author's information no previous study has been conducted to assess the residue load of commonly used insecticide in the buffalo liver across north India. The objective of this study was to assess the residue load of commonly used insecticides in buffalo liver across north India.

MATERIALS AND METHODS

Samples

A total of 100 buffalo liver samples were procured from various locations across North India for the detection and quantification of commonly used

insecticide residues. Samples were collected between December, 2014 to March, 2017 from Post Mortem Hall, Department of Veterinary Pathology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana) and from Delhi. Samples were collected between December, 2014 to March, 2017 from Haryana and Delhi. Following collection, all samples were immediately chilled and transported to the laboratory, where they were stored at -20°C until processing. Approximately 100 g of liver tissue from each animal was placed individually in polycarbonate vials for analysis.

Chemicals, reagents and laboratory wares

This study used pure standards of dichlorovos, chlorpyrifos methyl, fenitrothion, pirimiphos methyl, malathion, chlorpyriphos, quinalphos, ethion, edifenphos, α-HCH, β-HCH, γ-HCH, δ-HCH, α-endosulfan, β-endosulfan, aldrin, dicofol, λ-cyhalothrin, cypermethrin, fenvelrate, T-Fluvalinate, and deltamethrin with a purity of more than 98%. Ethyl acetate, methanol, petroleum ether (60–80), dichloromethane, acetone acetonitrile, and n-hexane were all analytical or HPLC grade solvents. All of the reagents, including sea sand, calcium silicate, celite 545, basic aluminium oxide, and anhydrous sodium sulphate, were of analytical quality.

Sample extraction and cleanup for organophosphates

Liver tissue sample matrices were treated using the procedure outlined by the Dutch Ministry of Public Health, Welfare, and Sports (AMPRF, 1996). For a consistent distribution of analytes, the blank, spiked, and field samples of buffalo liver (10 g) were thoroughly mixed separately and minced. In a mortar, the minced sample and sodium sulphate were combined to create a free-flowing powder. After adding 5 g of celite 545, 10 g of calcium silicate, 13 ml of acetone, and 125 ml of acetonitrile to this powder, it was mixed for two to three minutes. A partial vacuum was used to filter the extract via Whatman's filter paper No. 1 (100 mm diameter) over Buchner filter. The filtrate was dried in a rotating vacuum evaporator with a bath temperature of between 60 and 65 degrees Celsius.

After dissolving the evaporated residues in five millilitres of acetonitrile that had previously been saturated with n-hexane, the mixture was transferred to a separating funnel with a sixty-millilitre capacity that also contained five-millilitres of n-hexane that had previously been saturated with acetonitrile. Phases were allowed to separate after the funnel was shook forcefully for two minutes. After collecting and drying the lower (acetonitrile) fraction, it was reconstituted with 10 millilitres of ethyl acetate and subjected to gas chromatographic analysis.

Sample extraction and cleanup for organochlorines and synthetic pyrethroids

Liver tissue sample matrices were treated using the procedure outlined by the Dutch Ministry of Public Health, Welfare, and Sports (AMPRF, 1996). To ensure that the analytes were distributed uniformly, the blank, spiked, and field samples of muscle and liver (10 g each) were combined separately and minced. A free-flowing powder was produced by combining the minced sample with 5 g of sand and 10–20 g of sodium sulphate. This powder was put into thimbles with Whatman filter paper No. 1, inserted into the Soxhlet apparatus's extractor, and extracted for two hours using 150 millilitres of petroleum ether. In a rotary vacuum evaporator with a bath temperature of 60–65^{0}C, the extract was evaporated until it was completely dry. The gravimetric method was used to calculate and record the amount of extracted fat. After that, the residue was redissolved in enough petroleum ether to make a solution with 50 mg of fat per millilitre for additional cleanup.

To clean up the sample, two millilitres of residue-containing petroleum ether were run over a chromatographic column that had been pre-rinsed with petroleum ether and loaded with four grams of aluminium oxide. Next, 25 millilitres of petroleum ether were used to elute the column. After the elute was dried off and reconstituted with 10 millilitres

of n-hexane, gas chromatographic analysis was performed.

GC conditions

A Shimadzu gas chromatography model GC 2010 Plus equipped with auto sampler AOC-20i mounted on a split/splitless injector port connected to ^{63}Ni electron capture detector through Equity 5 capillary column (30 m x 0.25 mm I.D.) was used for analysis of OC and pyrethroid pesticides. Whereas, a programmed temperature vaporizer (PTV) injection port (manual injection) connected to nitrogen-phosphorous detector through Equity 1 capillary column (30 m x 0.25 mm I.D.) was used for analysis of OP pesticides.

In present study GC-MS tandem mass spectrometry (Agilent 7890A series) was used for confirmation of OC, Pyrethroid and OP residues detected in sample by GC. The operating parameters were: injection port temperature: 280 °C. Column: HP-5 MS (30 m x 0.32 mm i.d. x 0.25 µm film thickness) containing 5 percent diphenyl and 95 percent dimethyl polysiloxane. Oven temperature ramping was: 70 °C (2 min) ⟶ @ 25 °C min^{-1}⟶150 °C (0 min) ⟶ @ 15 °C min^{-1}⟶ 200 °C (0 min)⟶@ 8 °C min^{-1}⟶280 °C (2 min). Detector: Mass 7000 GC-MS/MS; detector parameters were: source temperature, 230°C; emission current, 35 µA; energy, – 70 eV; repeller voltage, 11 V; ion body, 12 V; extractor, –7.2 V; ion focus, –7.4 V; quadrupole one (MS^1) temperature, 150°C; quadrupole two (MS^2) temperature, 150°C. Gas flow rates: helium (carrier gas), 1 ml min^{-1} through column and 2.25 mL min^{-1} as collision flow/quench flow, nitrogen (collision cell), 1.15 ml min^{-1}.Other parameters: split ratio, 1:10; vacuum (high pressure), $2.23x10^{-05}$ torr; rough vacuum, $1.51x10^{+02}$ torr; injection volume, 2 µl.

Preparation of standard solutions

Ten grams of homogenised blank samples of liver tissue were fortified with a standard mixture of specific OC, SP, and OP pesticides at different concentrations (50, 100, 150, 200, and 250 ηg/g) for linearity and recovery investigations. After an hour at room temperature, the spiked samples were processed. Liver samples that had been stored were thawed to room temperature before being processed further.

Method validation

The validation research was conducted in accordance with the ICH Harmonized Tripartite Guidelines on Validation of Analytical Procedures: Methodology (ICH, 2005), taking into account the performance standards and other EU-recommended analytical technique requirements (2002).

RESULTS AND DISCUSSION

For OC and SP, the percentage Relative Standard Deviation (RSD) was less than 0.4265 percent for area and less than 0.0221 for retention time; for OP pesticides, it was less than 0.3477 percent for area and less than 0.0168 percent for retention time. Additionally, all of these RSD values were determined to be within the normal range, indicating that the instrumental response's reproducibility deviations were less than ideal. For nearly all concentrations, the coefficient of determination (r2) values discovered in the standard analytes of OC, SP, and OP studies were greater than 0.9, indicating a strong correlation between the analyte concentration and the area under the curve for the system's capacity to determine the same.

In the range of investigated values, or from 50 to 250 ppb, the standard calibration curves of the examined OC, SP, and OP pesticides showed a strong regression line (r2 greater than 0.916). The r2 values for each analyte were found to be greater than 0.9, indicating strong linearity, and the AUC increased proportionately with a matching increase in standard concentration.

The approach was able to detect the specified pesticide at a low enough level because the Limit of Detection (LOD) and Limit of Quantitation (LOQ) for each analyte were significantly below their corresponding MRLs. While the analyte-matrix recoveries of SP pesticides varied from 74.24 (T-fluvalinate) to 81.91 (deltamethrin), the analyte-matrix combinations of OC pesticides ranged from

Table 1: Method performance parameters for detection of OP pesticide residues

S.No.	Name of pesticide	Limit of Detection (in ppb)	Limit of Quantitation (in ppb)	Accuracy (Average recovery %)	Precision (Average CV %)
1	Dichlorovos	1.09	2.78	92.05	0.0176
2	Chlorpyrifos methyl	0.95	1.60	85.26	0.0188
3	Fenitrothion	1.87	4.67	96.27	0.0454
4	Primiphos methyl	0.36	0.53	85.74	0.0057
5	Malathion	0.81	2.04	87.77	0.0083
6	Chlorpyrifos	1.05	2.50	90.23	0.0079
7	Quinalphos	0.62	1.44	83.49	0.6476
8	Ethion	0.43	0.81	84.92	0.0049
9	Ediphenphos	0.17	0.28	88.89	0.0159

Table 2: Method performance parameters for detection of OC pesticide residues

S.No.	Name of pesticide	Limit of Detection (in ppb)	Limit of Quantitation (in ppb)	Accuracy (Average recovery %)	Precision (Average CV %)
1	Alpha HCH	2.348	5.649	82.00	0.71
2	Beta HCH	11.694	28.105	80.81	2.53
3	Gamma HCH	1.132	2.197	81.74	0.55
4	Delta HCH	2.513	5.151	78.63	0.93
5	Aldrin	0.654	1.444	80.45	0.31
6	Alpha Endosulfan	0.617	1.409	78.48	0.53
7	Beta Endosulfan	2.152	3.989	72.13	1.73
8	Dicofol	0.853	1.593	78.38	0.31

Table 3:Method performance parameters for detection of SP pesticide residues in meat

S.No.	Name of pesticide	Limit of Detection (in ppb)	Limit of Quantitation (in ppb)	Accuracy (Average recovery %)	Precision (Average CV %)
1	Lamda Cyhalothrin	1.184	2.906	78.48	1.079
2	Cypermethrin	3.732	8.181	77.30	1.604
3	Fenvelrate	1.399	2.874	78.26	1.446
4	T-Fluvalinate	0.950	2.373	74.24	1.546
5	Deltamethrin	3.966	8.039	81.91	1.550

Table 4:Percent presence and mean concentration (in ppb) of organophosphate pesticide residues in random buffalo liver samples

Pesticide	Mean±SD (n = 100)	Positive samples	Maximum sample concentration	Per cent positive[a]	Per cent Proportion[b]	MRLS by CAC[c]	Samples > MRL
Dichlorovos	0.89±3.40	8	23.7	8	15.39	10	2
Fenitrothion	0.37±3.67	1	36.7	1	6.38	50	0
Malathion	1.69±5.67	11	33.8	11	29.30	NA	—-
Chlorpyrifos	2.82±8.60	18	51.8	18	48.93	10	6

Total pesticide load of Organophosphate residues = 5.75 ppb, [a] Percentage of positive samples from total samples (100) analysed [b] Based on the mean residue levels, the contribution of a particular pesticide among total pesticide load detected in buffalo liver samples, [c] Maximum residue limits recommended by Codex Alimentarius Commission (2006)

72.13 (beta endosulfan) to 82.0 (alpha HCH). In beef tissue, the OP pesticide analyte-matrix recoveries varied from 83.49 (quinalphos) to 92.05 (dichlorovos). All pesticides, with the exception of beta endosulfan (119.68 percent), generally produced acceptable recoveries between 70 and 110 percent during the specified validation time as per EU legislation (EU, 2002). Method performance parameters (LOD, LOQ, Accuracy, Precision) for detection of, OP, OC and SP insecticides residues

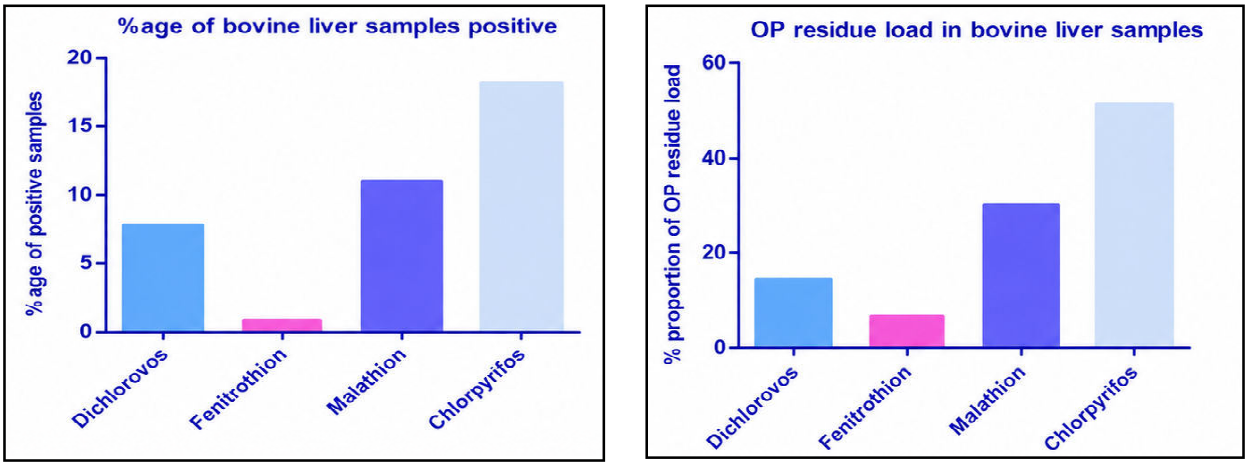

Fig. 1:Percentage of bovine liver samples positive and proportionate organophosphate pesticide residue load in buffalo liver samples

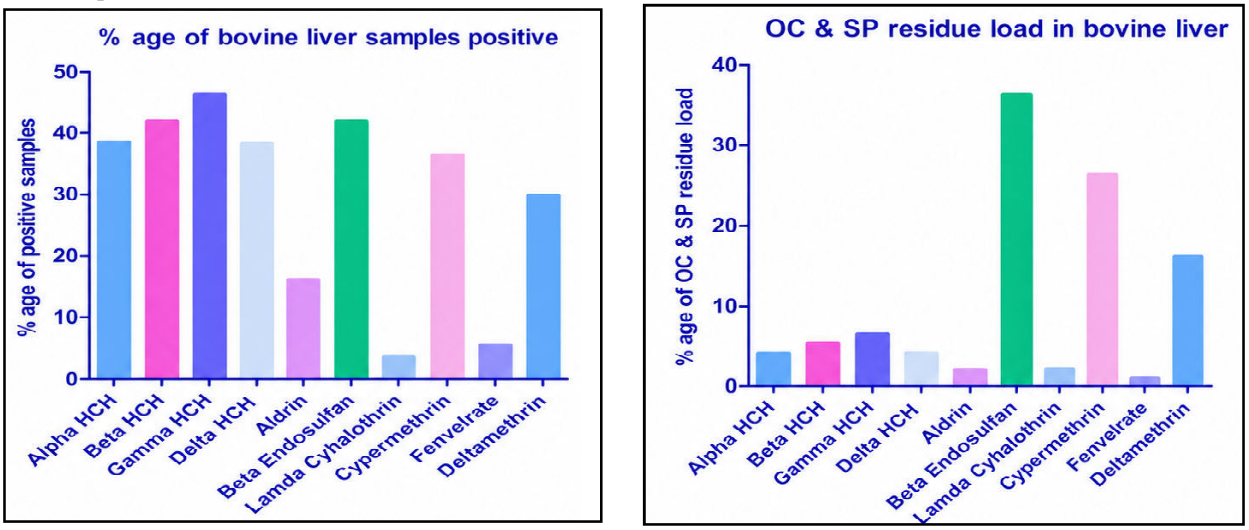

Fig. 2:Percentage of bovine liver samples positive and proportionate organochlorine and synthetic Pyrethroid pesticide residue load in buffalo liver samples

Table 5:Percent presence and mean concentration (in ppb) of OC and SP pesticide residues in random buffalo liver samples

Pesticide	Mean±SD (n = 100)	No. of Positive samples	Maximum sample concentration	Per cent positive[a]	Per cent Proportion[b]	MRLS by CAC[c]	Samples > MRL
Alpha HCH	1.80±2.74	19	9.4	38	3.34	NG	—
Beta HCH	2.45±3.42	21	13.8	42	4.54	NG	—
Gamma HCH	3.09±4.37	23	16.9	46	5.73	1	23
Delta HCH	1.86±2.76	19	9.5	38	3.44	NG	—
Aldrin	0.90±2.24	8	9.4	16	1.67	NG	—
Beta Endosulfan	19.54±34.70	21	136.7	42	36.25	100 (α+β)	4
Lamda Cyhalothrin	0.97±5.25	2	34.9	4	1.79	50	0
Cypermethrin	14.22±24.85	18	113.8	36	26.39	50	5
Fenvelrate	0.42 ±1.82	3	9.4	6	0.78	20	0
Deltamethrin	8.66 ±18.14	15	82.6	30	16.07	30	5

Total pesticide load of Organochlorine and Synthetic pyrethroid residues = 53.898 ppb, [a] Percentage of positive samples from total samples (50) analysed, [b] Based on the mean residue levels, the contribution of a particular pesticide among total pesticide load detected in buffalo liver samples, [c] Maximum residue limits recommended by Codex Alimentarius Commission (2006).

have been given in Table 1, 2 and 3 respectively. Extraction and analysis were used to evaluate the precision at five concentration levels of the recovery investigations. For all OC, SP, and OP pesticides, repeatability and intermediate precision values, represented as relative standard deviation (CV percent), were determined to be < 17.09,≤13.85, and≤0.6476 percent for liver tissue, respectively. Additionally, according to EU rules (EU, 2002), these values were found to be within an acceptable range of less than 23%.

Eighteen of the hundred buffalo liver samples that were examined for the presence of organophosphate pesticides were positive for chlorpyrifos, eleven for malathion, eight for dichlorovs, and one for fenitrothion. According to the Codex Alimentarius Commission, levels of dichlorovs and chlorpyrifos were above the MRL in two samples and six samples, respectively (2006). Buffalo liver samples had a total organophosphate pesticide concentration of 5.75 ppb. Table 4 and Fig. 1 show the results of organophosphate residue in buffalo liver samples. Fifty buffalo liver samples were examined for the presence of synthetic pyrethroid pesticide residues and organochlorines. According to the Codex Alimentarius Commission, levels of beta endosulfan and gamma HCH were found to be higher than the MRL in four and twenty-three samples, respectively (2006). The total amount of synthetic pyrethroid and organochlorine pesticide residue found in buffalo liver samples was 53.898 ppb. Table 5 and Fig. 2 show the results of organochlorine and synthetic pyrethroid residues in buffalo liver samples.

Only a small proportion of applied pesticides reaches the intended target organisms under field conditions. The majority is lost through processes such as spray drift, volatilization, runoff, leaching, photodegradation, and adsorption to soil particles, resulting in contamination of surrounding air, water, and soil environments (Elhamalawy *et al*., 2024; Sarker *et al*., 2024; Tang *et al*., 2025). The widespread environmental dispersion of pesticides results in exposure of organisms across multiple trophic levels through both direct contact and indirect dietary pathways. Persistent pesticide residues can be transferred through food webs, where bioaccumulation and, in some cases, biomagnification lead to elevated concentrations in higher trophic-level organisms, posing ecological and human health risks (Tang *et al*., 2025; Tison *et al*., 2024). Numerous monitoring studies have reported the occurrence of organochlorine, organophosphate, and pyrethroid pesticide residues in Indian agricultural soils. A survey conducted in northern India during 2002–2003 detected residues of HCH, DDT, endosulfan, chlorpyriphos, malathion, quinalphos, cypermethrin, and fenvalerate, reflecting the widespread use and persistence of these compounds in agroecosystems (Kadarla and Rajani, 2024; Kumari *et al*., 2008). Furthermore, pesticide residues detected in groundwater may exceed regulatory limits in intensively cultivated regions. These contaminants can migrate through environmental compartments, including soil, water, crops, livestock, and aquatic food webs, thereby contributing to dietary and environmental exposure pathways in humans (Beyuo *et al*., 2024; Zhou *et al*., 2025).

To protect public health and regulate pesticide contamination, regulatory authorities worldwide have established legally enforceable maximum residue limits (MRLs) for pesticides in food and, where applicable, drinking water. Systematic monitoring of pesticide residues is essential for verifying regulatory compliance, supporting international trade, conducting surveillance across food and environmental matrices, and improving understanding of the occurrence, fate, and potential risks associated with pesticide residues in the environment and food supply chain (Ayranci and Erkmen, 2026; Yang *et al*., 2024).

CONCLUSION

The present investigation revealed that concentrations of multiple pesticides in buffalo liver samples exceeded the MRLs recommended by the Codex Alimentarius Commission (2006). The mean organophosphate pesticide burden was recorded at 5.75 ppb, whereas the combined organochlorine and

synthetic pyrethroid load reached 53.898 ppb. Since several pesticide compounds are also excreted through milk, both buffalo meat consumers face appreciable insecticide exposure. These findings highlight an urgent requirement for establishing a comprehensive national residue monitoring framework to assess and control pesticide contamination in buffalo meat products.

Limitations of the study

More number of buffalo liver samples especially for Organochlorines and Synthetic Pyrethroids insecticides could have been included in this study. Likewise, more number of insecticides could have been included in this study.

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