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Analysis of buffalo liver samples for the presence of chlorpyrifos residues by using high performance liquid chromatography

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Abstract: Food safety is of great importance to all involved in the food chain. The problem of pesticide residues is one of the concerns for the consumers. In the present study, 254 buffalo liver tissue samples were collected from 4 slaughterhouses and analyzed both qualitatively and quantitatively for the presence of chlorpyrifos residues. The samples were processed by extraction with acetonitrile followed by sonication and liquid-liquid partition. The extracts were cleaned up by performing alumina column chromatography. Chlorpyrifos residues in the samples were detected and quantified by employing high performance liquid chromatography. High performance liquid chromatography (HPLC) was performed by using the isocratic mobile phase, consisting of acetonitrile and water at a ratio of 67:33 with a flow rate of 1 mL/min and a run time of 18 min. The detection wavelength was set at 202 nm with 360 nm as the reference wavelength. Chlorpyrifos residues were detected in 9.05% of the samples. Of these, only 0.78% of the liver samples were found to exceed the Codex maximum residue limit (MRL) for chlorpyrifos.

Key words: Residue, chlorpyrifos, buffalo liver tissue, HPLC, maximum residue limit

Introduction

Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phophorothioate) is widely used to improve the yield of agricultural produce in India. As per the Registration Committee under the Insecticides Act (1968) of India, as many as 181 pesticides have been registered for regular use in the country (1). As of today, 44 types of pesticides are manufactured in India and chlorpyrifos is in common use (2). The consumption of pesticides in India is 0.5 kg/ha, which is far less compared to usage in other countries, such as 7 kg/ha in the USA (1).

Chlorpyrifos is moderately toxic to humans. Poisoning from chlorpyrifos may affect the central

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nervous system, the cardiovascular system, and the respiratory system. It is also a skin and eye irritant. Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory depression, and slow heartbeat. Very high doses may result in unconsciousness, incontinence, and convulsions or fatality. There have been reports on the effects of chlorpyrifos on the reproductive (3) and endocrine systems (4).

India has the world's largest buffalo population (97.9 million), which is claimed to be 56% of the world buffalo population. Of the total Indian meat production, 25% is contributed by buffaloes, estimated to be 1.483 million t (5), while 70% of the total meat exported from India is buffalo meat, estimated to be 0.344 million t (6).

The liver is an organ of biotransformation and metabolism. Generally, any chemical that enters the body has to pass through the liver in order to get activated or to get excreted from the body. In the liver, chlorpyrifos undergoes metabolism by oxidative and hydrolytic enzymes (7-11).

Several methods have been developed for the determination of chlorpyrifos residues using chromatography (12-19). The sensitivity of the method standardized by Pradeep et al. (19) is 0.02929 µg/g, which is less than the Codex MRL of chlorpyrifos in buffalo meat. The recovery percentage is 86.17 ± 4.09%.

The present study was carried out to investigate the status of chlorpyrifos residues in buffalo liver samples collected from 4 local slaughterhouses by employing the method standardized by Pradeep et al. (19).

Materials and methods

Chemicals

HPLC-grade solvents such as acetonitrile, water, and dichloromethane, and GR-grade chemicals such as alumina (aluminium oxide, neutral activity I-II grade) and sodium sulfate were used. Technical grade chlorpyrifos (Sigma-Aldrich Co.) of 98.5% purity was used for the standardization.

Apparatus

A homogenizer (Polytron®), a refrigerated centrifuge (Multifuge 1 S-R®), an ultrasonicator (Soniprep®), and a vacuum manifold pump were used.

HPLC system

A PerkinElmer® (model series 200) system comprising a quaternary LC pump, an autosampler with a Rheodyne injector having a 200-µL loop, a diode array detector, and a Peltier column oven was used.

Column

A LiChroCART® 250-4/LiChrospher® 100 RP-18e endcapped (250 × 4 mm, with particle size of 5 µm) column was used.

Mobile phase

The mobile phase consisted of acetonitrile and water at the ratio of 67:33. The flow rate was 1 mL/min and the run time was 18 min.

Stock standard solution

The stock standard solution of chlorpyrifos at a concentration of 100 µg/mL was prepared by dissolving 10 mg of standard chlorpyrifos in 100 mL of acetonitrile.

Collection of samples

From various parts of northern India, buffaloes are being slaughtered at Barreily, Haldwani, Kichha, and Baheri slaughterhouses. A total of 254 buffalo liver samples were collected from these 4 slaughter houses (Bareilly: 107, Haldwani: 87, Kichha: 42, and Baheri: 18) belonging to Uttar Pradesh and the Uttarakhand state of India. The samples were placed in clean polyethylene bags, labeled properly, and transported to the laboratory in a thermo-cool container jacketed with ice packs. The samples were processed and analyzed for the presence of chlorpyrifos residues within 24 h.

Extraction and cleanup of the samples

The extraction and cleanup of chlorpyrifos residues from the liver tissue samples were carried out following the methods of Bottomley and Baker (13) and Pradeep et al. (19) (Figures 1 and 2).

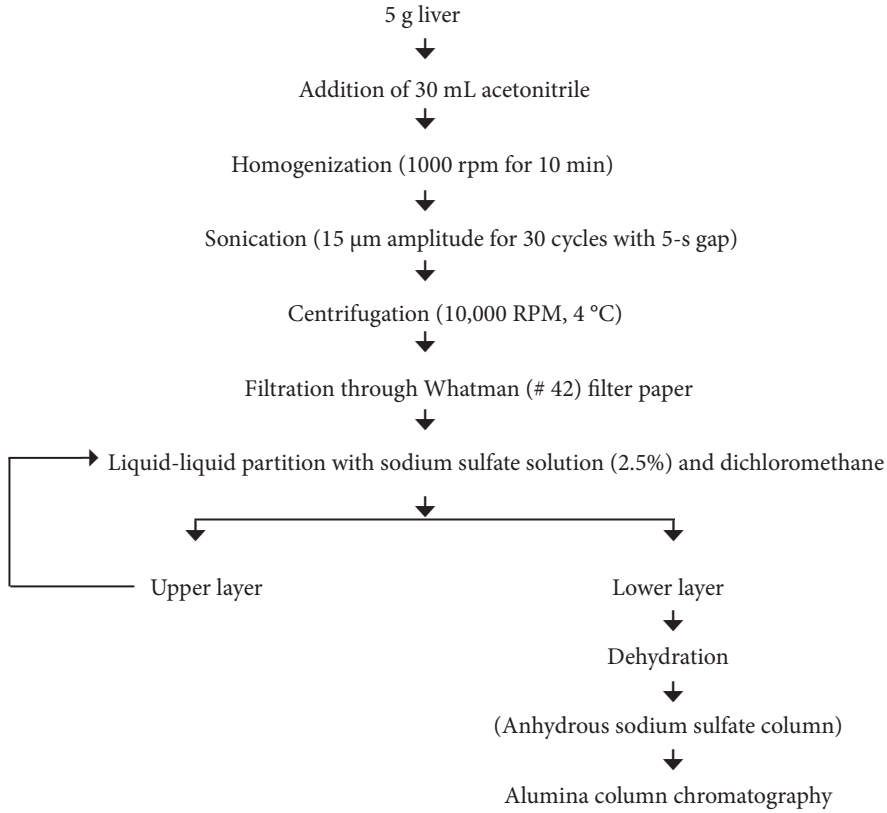


Figure 1. Extraction of chlorpyrifos residues from buffalo liver.

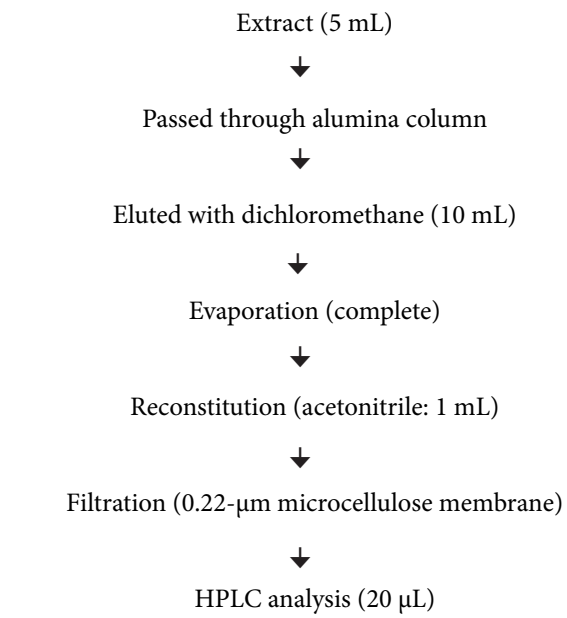


Figure 2. Cleanup of chlorpyrifos residues from extract of buffalo liver.

Detection of chlorpyrifos residues by HPLC

The content of the beaker after extraction and cleanup was reconstituted in 1 mL of acetonitrile and filtered through a 0.22-μm Millipore membrane filter. A volume of 20 μL of this reconstituent was injected into the column for the HPLC run. Chromatography was performed by using a diode array detector (DAD) at a detection wavelength of 202 nm with 360 nm as the reference wavelength. The temperature was kept constant at 40 °C. The run and analysis of samples was carried out by using TotalChrom software.

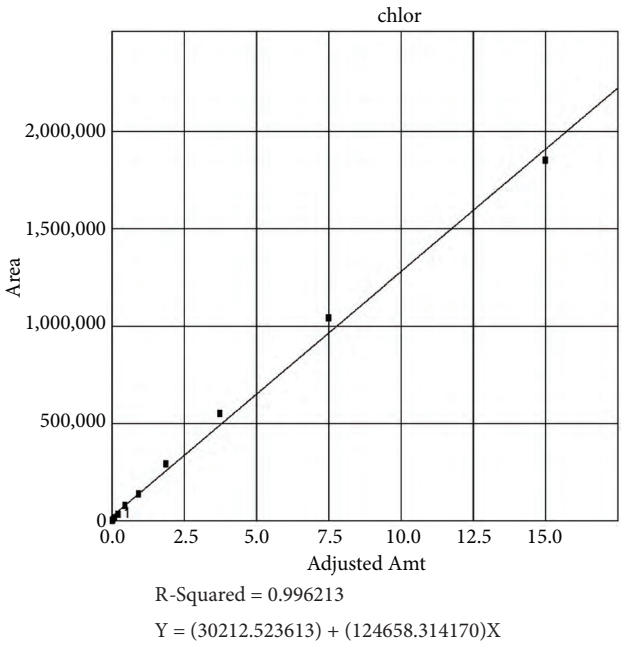
Quantification of chlorpyrifos residues

For the quantification of chlorpyrifos residues in the liver tissue samples, a standard calibration graph was obtained by running different dilutions of the standard chlorpyrifos. A volume of 150 μL of stock solution was diluted with 850 μL of acetonitrile so as to get a working standard solution with a concentration of 15 μg/mL. This working standard solution was further diluted to get 2-fold dilutions, i.e. 15.00, 7.50, 3.75, 1.875, 0.9375, 0.46875, 0.234375,

0.117187, 0.05859, and 0.029295 µg/mL (Figure 3). An aliquot of 20 µL of these different dilutions was thrice injected into the column for HPLC analysis, and a standard graph was obtained by plotting the concentration versus the peak area (average).

Recovery analysis

The buffalo liver samples found to be negative for chlorpyrifos residues were fortified with different known concentrations of standard chlorpyrifos and subjected to extraction, cleanup, and detection similar to the test samples to determine the percentage recovery (16). The recovery percentage obtained was used for the estimation of the correction factor. This correction factor was then used to calculate the actual concentration of chlorpyrifos residues in the test samples. The formulas used were as follows.



S. No.	Concentration (ppm)	Area
1	0.02929	5421.12
2	0.05859	13,385.15
3	0.11718	21,141.89
4	0.23437	40,015.87
5	0.46875	82,547.02
6	0.93750	139,975.46
7	1.87500	294,571.19
8	3.75000	554,712.01
9	7.50000	1,041,206.79
10	15.00000	1,845,245.20

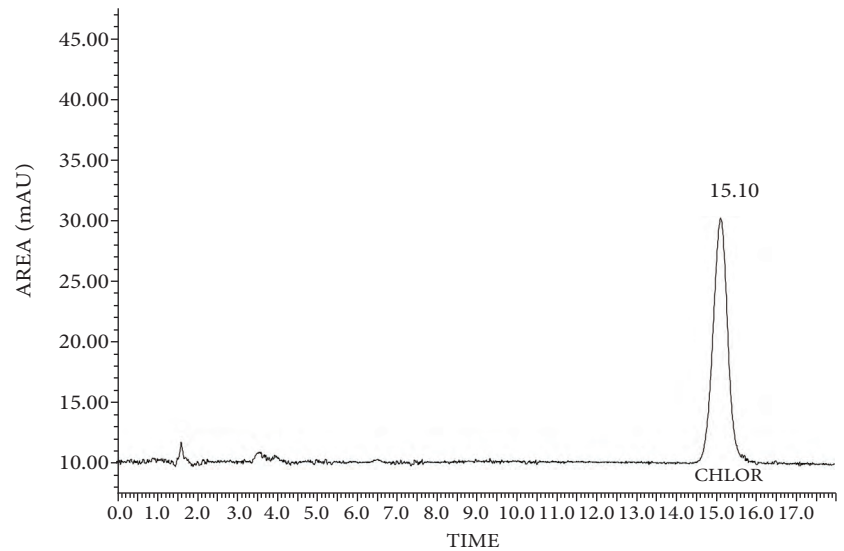


Figure 3. Calibration curve and a HPLC chromatogram of standard chlorpyrifos.

$$(1)\ \text{Recovery}\ (\%) = \frac{N(\Sigma xy) - (\Sigma x)(\Sigma y)}{N(\Sigma x^2) - (\Sigma x)^2} \times 100,$$

where x = amount of standard pesticide, N = number of observations, and y = amount of pesticide detected by the standardized method.

$$(2)\ \text{Correction factor} = \frac{100}{\text{Recovery \%}}.$$

Statistical analysis

In the present study, mean as a measure of central tendency, range, standard deviation (SD) and coefficient of variation (CV) as a measure of dispersion, and correlation coefficient and regression coefficient as a measure of relationship were employed for the statistical analysis of the data.

Results

In order to determine the concentration of chlorpyrifos in the liver samples collected from the 4 slaughterhouses, the standard calibration graph was used. The recovery percentage (±SE) obtained was 86.17 ± 4.09%, and the correction factor was 1.160. Figure 3 shows the calibration curve and a HPLC chromatogram of standard chlorpyrifos, and Figure 4 shows HPLC chromatograms (superimposed) of different dilutions (ppm) of standard chlorpyrifos.

Residual concentrations of chlorpyrifos in buffalo liver samples

In the present study, of the samples analyzed, a total of 23 (9.05%) of the liver tissue samples were found to be positive for residues of chlorpyrifos. The residual concentrations of chlorpyrifos in the liver tissue samples were found to range from 0.0042 to 1.1368 µg/g. Figure 5 shows a liver sample collected from the Haldwani slaughterhouse that exceeded the Codex MRL for chlorpyrifos residues. The location-wise distribution of percentage of positive samples and percentage of positive samples exceeding the Codex MRL of residues is depicted in the Table and Figure 6.

Discussion

The method developed by Pradeep et al. (19) is HPLC-based. HPLC-based detection methods are better than any other methods for the detection of pesticide residues in food. The above method was found to be sensitive and valid, as the recovery percentage was very good, 86.17 ± 4.09%. Hence, this method was used in the present study.

Organizations and authorities of different ecological zones of the world and the World Health

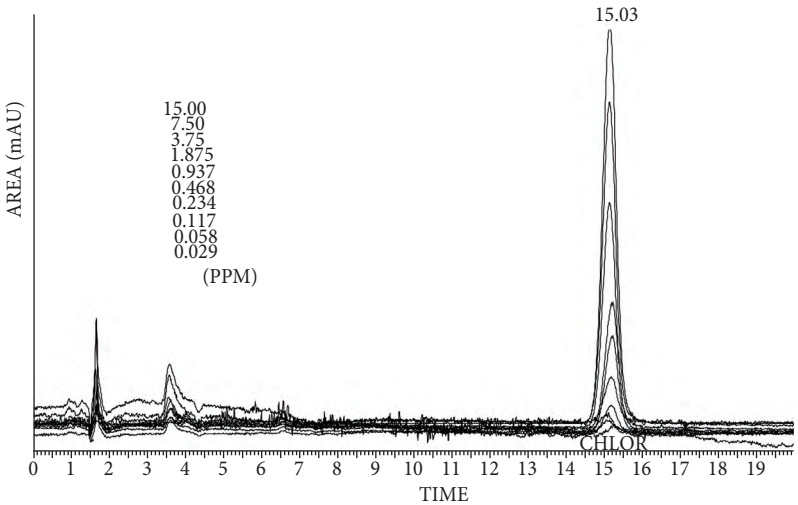


Figure 4. HPLC chromatograms (superimposed) of different dilutions (ppm) of standard chlorpyrifos.

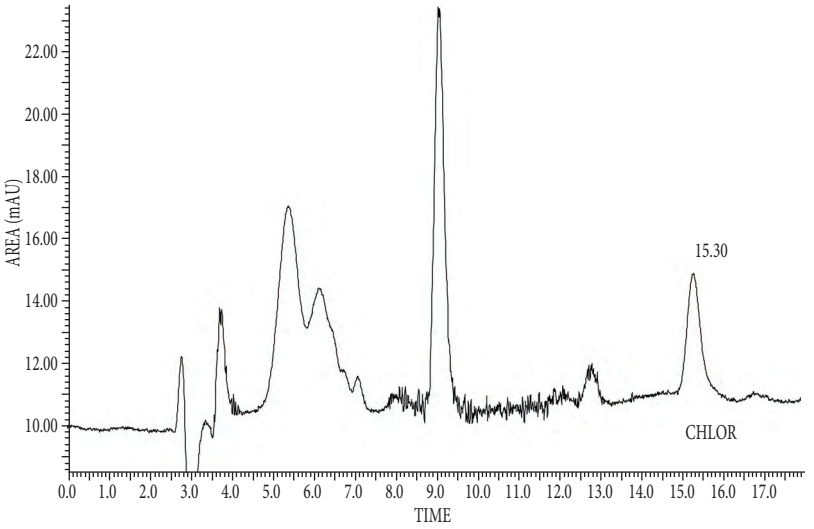


Figure 5. HPLC chromatogram of a liver sample from Haldwani slaughterhouse that was found to be positive for chlorpyrifos residues.

Table. Location-wise distribution of buffalo liver samples positive for chlorpyrifos residues.

Location	No. of samples analyzed	No. of samples detected positive (%)	Concentration range (µg/g)	Mean residual concentration (µg/g) ± SE	No. of samples exceeding Codex MRL* (%)
Bareilly	107	6 (5.6%)	0.04640-0.4980	0.168983 ± 0.014829	0
Haldwani	87	15 (17.24%)	0.00420-1.1368	0.338633 ± 0.058400	2 (2.29)
Kichha	42	2 (4.76%)	0.03410-0.0714	0.052750 ± 0.002870	0
Baheri	18	ND	—	—	—
Total	254	23 (9.05%)	0.00420-1.1368	0.269517 ± 0.028700	2 (0.78%)

*The MRL for chlorpyrifos in buffalo meat set by Codex is 1 ppm.
ND: Not detected.
SE: Standard error.

Organization (Codex) have set MRLs for each pesticide in every food commodity. For the export and import of food commodities, we must observe and analyze the food samples with respect to the MRL for chlorpyrifos in buffalo meat. When we compared the concentration of chlorpyrifos residues in the positive buffalo meat samples with the MRL in beef, though the number of samples positive for chlorpyrifos residues was greater, only 2 of the 23 positive samples (0.78% of the total samples) exceeded the Codex MRL (20) value of 1 µg/g. When we compared this with the MRL value of 1 µg/g set by Canada (21) and Korea (22), only 2 samples exceeded that level. Meanwhile, 4 samples exceeded the MRL value of 0.5 µg/g of chlorpyrifos in beef set by Japan (23), 4 samples exceeded the MRL value of 0.5 µg/g

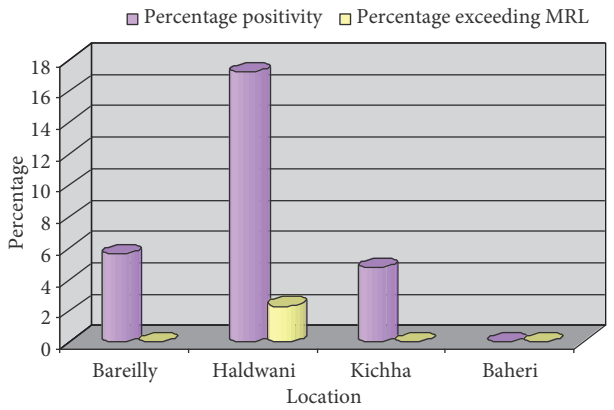


Figure 6. Location-wise percentage distribution of buffalo liver samples positive for chlorpyrifos residues.

of chlorpyrifos in beef fat (no limit has been set in beef) set by Australia (24-26), and 6 meat samples were found to exceed the MRL value of 0.1 µg/g set by India (1). The United States of America (27) and the European Union (28) have not set MRLs for chlorpyrifos residues in beef.

The reason for the high number of samples found to be positive for chlorpyrifos residues collected from the Haldwani slaughterhouse could be the use of large quantities of chlorpyrifos, as evidenced by the survey conducted by Simon (29). This can be further supported by the reports of chlorpyrifos residues in buffalo muscle samples collected from the same slaughterhouse by Pradeep et al. (30) and in feed (17.5%) and fodder (4%) samples collected from this region by Nagappa (31). However, meat samples from the Tarai region of northern India are being regularly tested for the presence of chlorpyrifos residues in our laboratory. The meat that exceeds the Indian limit is advocated for condemnation.

Out of the 254 liver tissue samples tested, 9.05% were found to contain the residues of chlorpyrifos. These findings reveal that there is injudicious use of chlorpyrifos, especially in the areas surrounding the Haldwani slaughterhouse.

However, only 0.78% of the liver tissue samples were found to exceed the MRL (1 µg/g) of chlorpyrifos in buffalo meat set by Codex, Japan, Korea, and Canada.

In view of the above, there is a need for further work on the monitoring of pesticide residues regularly all over the country. The reason for the high percentage of positivity for pesticide residues could be the unscientific farming of animals and the indiscriminate and injudicious use of pesticides by the farmers. Therefore, the farmers of this region should be educated about judicious use of pesticides and adoption of good agricultural practices, organic farming, and integrated pest management.

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