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Assessment of Pesticide Residues in Bovine Milk and the Human Health Risk in Punjab, India

Abdulrahman Mohammed[*1,2], Rabinder Singh Aulakh[2] and Jasbir Singh Bedi[2]

[1]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno Nigeria.

[2]Centre for One Health, Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, Punjab, India

* Corresponding author: E-mail address: abdulrahmanm@unimaid.edu.ng
Phone: +2348032844440

ABSTRACT

Status of pesticide residues in bovine milk in Punjab, India, was determined; and human health risks resulting from consumption of contaminated milk were assessed. Two hundred and two samples (202) collected from four districts of Punjab were analyzed by gas chromatography. The pesticide residues detected in 10.9% of the samples included parathion, malathion, quinalfos, profenofos, ethion, p,p' DDD - a synthetic organochlorine insecticide and a metabolite of the pesticide DDT (Dichlorodiphenyltrichloroethane), p,p' DDE (4,4'-Dichlorodiphenyldichloroethylene), lindane, cypermethrin, cyhalothrin and permethrin, with p,p' DDE contributing 21% of total pesticide load. Two samples contained profenofos levels above Maximum residue limit (MRL), while p,p' DDD, p,p' DDE and lindane levels were above MRL in one sample each. Hazard Index for all pesticides were far below 1 for both the lower-bound (LB) and upper bound (UB) scenarios. The cancer risk from consumption of milk contaminated with lindane or p,p DDD or p,p DDE for both adults and children at both LB and UB scenarios were also found to be within the safe limits.

Key words: Pesticide residues, Bovine milk, Human health risk, Maximum residue limit, DDD

1. INTRODUCTION

Surveys conducted in many countries to monitor pesticide residues reveal widespread contamination of milk with DDT, Hexachlorocyclohexane (HCH) and other pesticides. In India, milk and dairy products constitute the primary sources of dietary exposure to DDT and HCH [1]. Numerous epidemiological and toxicological investigations have identified correlations between pesticide exposure and a range of human health conditions, including neurodegenerative disorders, endocrine disruption, various cancers, hepatic and renal diseases, diabetes, immune system dysfunctions, and reproductive disorders [2-6]. Milk contamination with pesticides may occur due to animals consuming contaminated feed, fodder, and water, as well as through the administration of pesticides on the animals and their environment for pest management [7]. A survey conducted to assess the extent of organochlorine pesticide contamination in bovine milk in Jaipur city, Rajasthan, revealed that all milk samples contained DDT and its metabolites, various isomers of HCH, heptachlor, and aldrin [8]. The percentage DDT contamination in milk samples from Punjab in 1999, 2000, and 2001 were 0%, 16.6%, and 0%, while lindane contamination within this period conformed to100%, 50%, and 20%, respectively [9]. Analysis of 312 bovine milk samples from Punjab, India, revealed that chlorpyrifos, DDT, and γ-HCH (lindane) were the major contaminants. Also detected were residues of β-endosulfan, endosulfan suphate, cypermethrin, cyhalothrin, fenvalerate, deltamethrin, malathion, profenofos, and ethion in milk samples [10]. In India, restricted use of DDT for malaria control is permitted, which may account for its occurrence in milk samples. The assessment of human health risks of non-cancer and cancer hazards was determined using both lower-bound (mean residue levels) and upper-bound (95th percentile levels) restrictions. Cancer and non-cancer risks were observed to be under the limits established by the United States Environmental Protection Agency for children as well as adults at the LB; however, children faced heightened exposure risks for DDT and HCH at the 95th-percentile UB level]10]. Conversely, another study [11] examined 64 samples each of exotic cow and buffalo milk from Jaipur, Rajasthan and found no pesticides in milk at the detection limit of 0.01 mg/kg. The present study was conducted to assess the status of contamination of milk with pesticide residues, including organophosphates and synthetic pyrethroids, and to analyze the associated human health risks from dietary pesticide exposure.

2. MATERIALS AND METHODS

2.1 Sampling

A total of two hundred (200) milk samples were collected from Ludhiana, Sangrur and Moga districts of Punjab in 2017. Milk samples were aseptically collected from dairy farms to eliminate the potential for external contamination during collection and transit. All samples were collected in screw-capped bottles and stored at 4°C until analyzed. High-purity analytical standards of organochlorines (OCs), organophosphorous (OPs) and synthetic pyrethroids pesticides (SPs)) were procured from Sigma-Aldrich, USA and Rankem Ltd., Mumbai, India, and used for recovery tests and quantification of residues in samples.

2.2 Extraction and clean-up of residues in bovine milk samples

Pesticide residues from milk samples were extracted using the method described by Battu [12]. Samples were equilibrated to room temperature and meticulously homogenized prior to analysis commencement. Five millilitres (5 ml) of sample were extracted and thoroughly combined with 20 grammes of pre-washed and freshly activated silica gel, together with 20 grammes of anhydrous sodium sulphate, to create a free-flowing powder. The mixture was loaded into a glass extraction column (60 x 2 cm i.d.) containing 40 ml of dichloromethane above a glass wool plug and permitted to stand for 90 minutes. The solvent was eluted dropwise, initially with dichloromethane from the column, followed by 150 ml of a Dichloromethane : acetone (1:2 v/v) mixture. The extracted material was condensed to approximately 2 ml under vacuum utilising a rotary evaporator at 40 °C. Subsequently, 15 ml of n-hexane was introduced, concentrated, and the final reconstitution was executed in a 3 m n-hexane: acetone (1:1) mixture for analysis using gas chromatography (GC) equipped with an electron capture detector (ECD) and a flame thermionic detector (FTD). The extraction and quantification method for pesticide residues in milk samples was validated by determining the recovery from samples spiked with known quantities of pesticide standards at 0.1 mg/kg. The mean recovery values of spiked samples ranging from 86.53% to 90.03% were recorded. The method's reproducibility was assessed by calculating the relative standard deviation of the recovery values, which were determined to be under 10%. The detection limits varied from 1.0 ng g-1 for organochlorine pesticides (OCPs) to 2.0 ng g-1 for synthetic pesticides (SPs) and organophosphates (OPs).

2.3 Residue Estimation

The residues in purified extracts were measured using gas chromatography. The ECD and FTD were employed for organochlorine and organophosphorus compounds, respectively. A 2 μl aliquot of the purified extract was loaded into the gas chromatograph. The GC Solution 2010 software (Shimadzu, Japan) on the PC was utilized for the integration and calculation of signals. The chemicals were identified and quantified by comparing the retention time and peak height/area of the sample chromatograph with those of standards analyzed under identical operating conditions. The formula employed for quantifying residues was: ng of standard injected multiplied by the peak area of the sample multiplied by the final volume of the sample extract.

Residues (mg/kg) = (Peak area of the standard x μl of sample injected) / Initial volume of the sample.

2.4. Data analyses

Statistical analyses were conducted with Microsoft Excel and SPSS version 16.0. Data generated were subjected to one-way analysis of variance (ANOVA) and Duncan post hoc test, mean differences at $p<0.05$ were considered significant. Results were expressed as mean ± standard error of the mean (s.e.m) and in tables, charts and percentages.

2.5 Human Health Risk Assessment from consumption of milk

To assess the health risks for humans as a result of consumption of contaminated milk, Estimated Daily Intake (EDI) was calculated to determine the magnitude of exposure to pesticides and the data were evaluated under two contamination scenarios. In the first scenario, the lower-bound (UB) scenario involved results for samples in which pesticides were not detected but considered as zero; the second scenario corresponds to the 95th-percentile upper-bound (UB) which entailed the setting of undetected results to the detection limit. The LB tends to underestimate contamination and exposure levels, while the UB results in overestimation [10].

Initially, dietary pesticide exposure was evaluated by calculating the estimated daily intake (EDI) values of pesticides identified in milk samples, in accordance with the guidelines established by the Food and Agricultural Organization/World Health Organization [13] for predicting dietary intake of pesticide residues. The estimated daily intake (EDI) of pesticides was derived from the subsequent equation:

$$EDI = C_{milk} \times I$$

Where C_{milk} denotes the concentration of pesticide residues in milk; I represents the daily availability of milk to an individual, measured in grammes; Exposure was assessed on a per capita basis, with milk consumption in Punjab quantified at 944 g/person/day [14]; EDIs were documented in microgrammes of pesticide per kilogramme of body weight each day. The estimations of food intake of pesticide residues were compared with the Acceptable Daily Intake (ADI) limits established by FAO/WHO [15].

The potential hazards of exposure were assessed for both carcinogenic and non-carcinogenic effects based on the predicted food consumption of pesticide residues as outlined by the USEPA [16] and detailed by Bedi *et al*. [10]. For non-cancer risk, Hazard Ratios were calculated by dividing the average daily exposure by the benchmark concentration (BMC) for non-carcinogenic effects, which are based on the reference dose (RfD) established by the USEPA Integrated Risk Information System for each pollutant. The oral RfD is predicated on the premise that thresholds exist for specific hazardous consequences, including cellular necrosis. It is quantified in milligrammes per kilogramme per day. The RfD is an estimate, with potential uncertainty of almost an order of magnitude, of daily exposure for the human population, including sensitive subgroups, that is expected to pose no significant risk of adverse effects over a lifetime [16].

$$\text{Hazard Ratio (HR)} = \frac{\text{Average daily exposure}}{\text{Bench mark concentration}}$$

The average daily exposure level for each contaminant within a population was determined using the following method:

Mean daily exposure µg/kg bw = Milk consumption (g/kg bw) x contaminant concentration (µg/kg), where bw denotes body weight. A benchmark concentration (BMC) denotes a daily concentration within which there is a significant likelihood of no detrimental health effects. An HR greater than 1 indicates a significant mean exposure level above the BMC, hence presenting a possible risk to human health [17].

The USEPA and IARC have listed most organochlorine pesticides present in milk and dairy products as probable carcinogens. The cancer risk was determined by multiplying the lifetime average exposure to a chemical by its cancer potency factor. The cancer potency values for the present analysis were sourced from United States Environmental Protection Agency (US EPA). The EPA has historically proposed an acceptable cancer risk range of 10^{-6} to 10^{-4}, with 1 x 10^{-5} being the standard recommendation. The carcinogenic risk was determined using the following equation, assuming a 70-year lifespan for adults.

$$\text{Risk} = ADD \times ED \times (1/70) \times CPF \times 10^{-6}$$

ADD represents the average daily dosage in ng kg^{-1}bw per day, whereas ED denotes the exposure length, corresponding to the age of the exposed individual (in this study, 60 years was utilised for adults and 10 years for children). CPF denotes the cancer potency factor, expressed in mg kg^{-1} bw per day^{-1}.

RESULTS

3.1 Levels of pesticide residues in bovine milk samples

Twelve different pesticides were detected in 10.9% of the 202 milk samples analyzed. Residues of parathion, malathion, quinalfos, profenofos and ethion, p,p' DDD, p,p' DDE, endosulfan, lindane (γ-HCH), cypermethrin, cyhalothrin and permethin were found in the samples (Table 1). Generally, the level of contamination was found to be very low in terms of frequency of occurrence. Among organophosphates, the most predominant pesticide, malathion, was found in only 2.5% of the samples. Although mean residue level of all the pesticides detected in milk in this study were lower than their respective MRLs, two individual samples exceeded the MRLs for profenofos, and one each for p,p' DDD, p,p' DDE and lindane, respectively.

Table 1. Levels of pesticide residues (μgkg^{-1}) in bovine raw milk (n=202) from Punjab

Pesticide	Mean ± SD	Positive (%) samples	% Proportion[a]	MRLs	Samples above MRL
Parathion	0.5 ± 6.8	1 (0.5)	8.1	Undefined	-
Malathion	1.0 ± 7.1	5 (2.5)	16.1	Undefined	-
Quinalfos	0.2 ± 2.6	1 (0.5)	3.2	Undefined	-
Profenofos	0.3 ± 3.1	2 (1.0)	4.8	10	2
Ethion	0.2 ± 1.5	3 (1.5)	3.2	Undefined	-
p, p' DDD	0.2 ± 1.8	2 (1.0)	3.2	20	1
p, p' DDE	1.3 ± 18.4	1 (0.5)	21.0	20	1
Endosulfan	0.2 ± 2.3	2 (1.0)	3.2	10	0
Lindane	0.3 ± 4.4	1 (0.5)	4.8	20	1
Cyperme-thrin	0.2 ± 2.5	1 (0.5)	3.2	50	0
Cyhalothrin	0.7 ± 5.0	4 (2.0)	11.3	200	0
Permethrin	1.1 ± 10.8	2 (1.0)	17.7	undefined	-

[a] Based on the mean residue levels, the contribution of a particular pesticide among total pesticide residue load detected in milk samples

[b] Maximum residue limits (MRL) recommended by Codex Alimentarius Commission (CAC (μgkg^{-1} body weight))

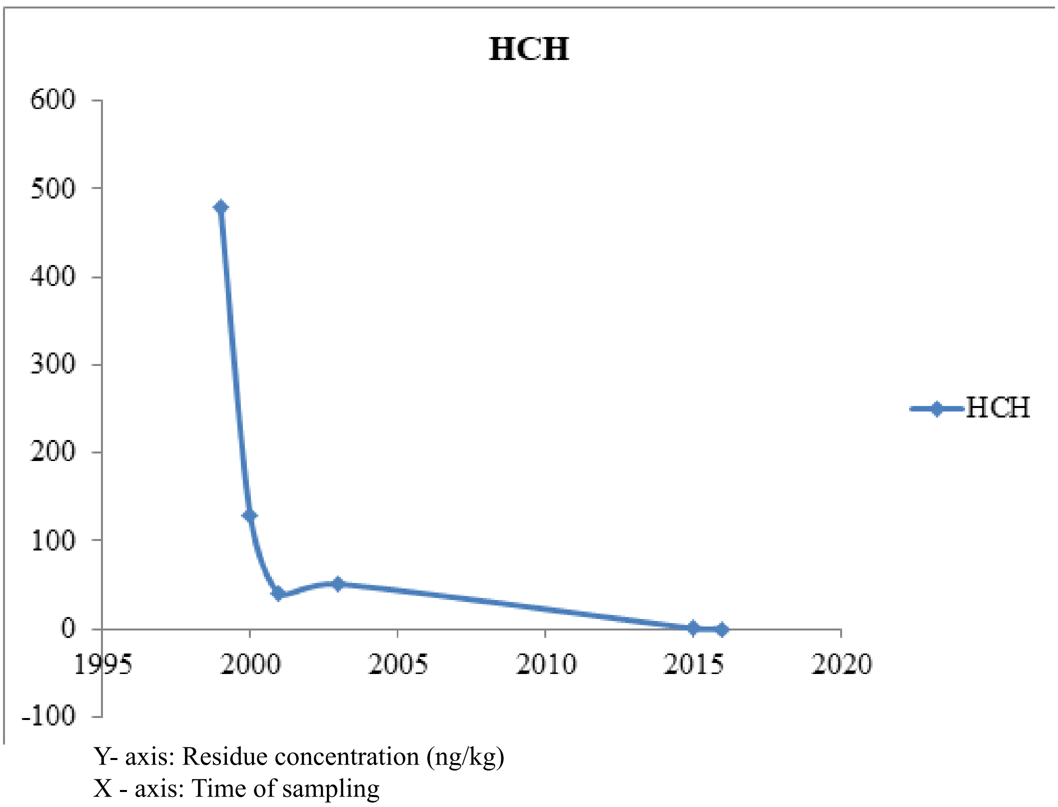
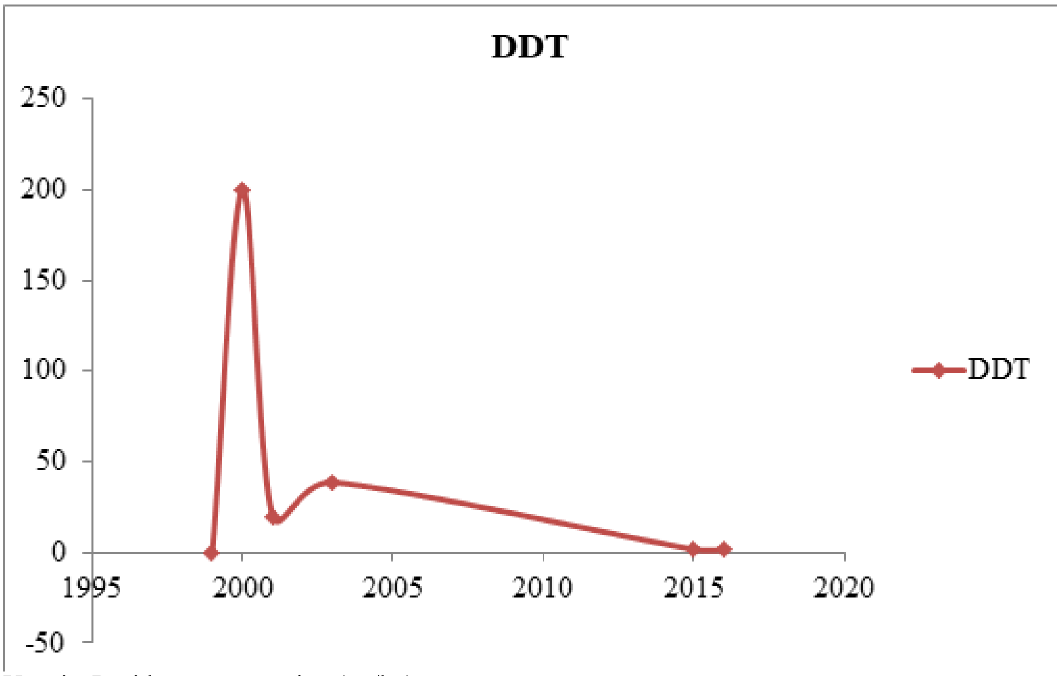


Fig. 1: Mean concentration of HCH residue in milk from Punjab Showing decrease in levels from 1999-2016



Y- axis: Residue concentration (ng/kg)
X - axis: Time of sampling

Fig. 2: Mean concentration of DDT residues in milk from Punjab from 1999-2016

3.2 Human health risk assessment from consumption of milk

Table 2 shows the estimated average daily dietary intake (EADDI) (µg/d) of pesticide residues through consumption of milk at LB (mean) and UB (P95) approaches. For all the pesticides, estimated daily intakes of pesticide residues fell far below the acceptable daily intake (ADI) in both the lower-bound and upper-bound scenarios.

Table 2. Estimated average daily dietary intake (EADDI) (µg/d) of pesticide residues through consumption of milk at LB (mean) and UB (P95) approaches

Pesticide	ADI[a]	TADI[b] Adults (µg/day)	TADI[c] children (µg/day)	EADDI Mean LB (µg/day)	EADDI 95_UB (µg/day)
Parathion	4	240	40	0.5	2
Malathion	300	18000	3000	1.0	2
Quinalfos	20	1200	200	0.2	2
Profenofos	30	1800	300	0.3	2
Ethion	2	120	20	0.2	2
p, p' DDD	10	600	100	0.2	1
p, p' DDE	10	600	100	1.3	1
Endosulfan	6	360	600	0.2	1
Lindane	5	300	500	0.3	1
Cypermethrin	20	1200	200	0.2	2
Cyhalothrin	20	1200	200	0.7	2
Permethrin	50	3000	500	1.1	2

[a]Acceptable Daily Intake (ADI) recommended by Codex Alimentarius Commission (CAC (µgkg^{-1} body weight))

[b] TADI (total ADI) value for adults was multiplied by average body weight of 60 kg

[c]TADI value for children was multiplied by average body weight of 10 kg

EADDI= estimated average daily dietary intake

P95= 95th percentile

3.3 Assessment of non-cancer risk

Hazard Index (HI) was calculated for all the pesticides detected in this study and the results are presented in table 3. The results showed that hazard indexes (HIs) for all pesticides studied were far below 1 for both the lower-bound (LB) and upper bound (UP) scenarios. It could, therefore, be extrapolated that exposure to pesticide through the consumption of milk in the study location did not pose cancer risk either to children or adults at both the LB and UP situations.

Table 3. Hazard Index for pesticide residues from milk in adults and children at LB (mean) and UB (P95) approaches

Pesticide	RfD[a]	Adults Mean LB	Adults P95_ UB	Children Mean LB	Children P95_UB
Parathion	6.0	0.00	0.00	0.01	0.03
Malathion	20.0	0.00	0.00	0.01	0.01
Ethion	0.5	0.01	0.01	0.04	0.40
p, p' DDD	0.5	0.01	0.01	0.04	0.20
p, p' DDE	0.5	0.04	0.09	0.26	0.20
Endosulfan	6.0	0.00	0.00	0.00	0.02
Lindane	0.3	0.02	0.06	0.10	0.33
Cypermethrin	10.0	0.00	0.00	0.00	0.02
Cyhalothrin	5.0	0.00	0.00	0.01	0.04
Permethrin	50	0.00	0.00	0.00	0.00

[a] Reference dose (RfD) values obtained from USEPA (µgkgbw/ day); P95= 95th percentile

3.4 Assessment of cancer risk

The risks of cancer to the public from consumption of milk contaminated with pesticides in the present study are summarized in table 4. The cancer risk from consumption of milk contaminated with lindane, p,p DDD and p,p DDE for both adult and children at both lower-bound (LB) and upper-bound (UB) scenarios were found to be within the safe range. In the LB scenario, the cancer risk for adult from lindane was 5.57 x 10^{-6} while the risk is 5.57 x 10^{-5} at the UB level.

Table 4. Estimated cancer risk associated with exposure to pesticides from milk at LB (mean) and UB (P95) approaches

Pesticide	USEPA[a] classification	CSF[b]	Mean LB Adult	P95_UB	Mean LB Children	P95_UB
Lindane	B2	1.3	5.57E-06	1.86E-05	5.57E-06	1.86E-05
p, p' DDD	B2	0.24	6.79E-07	3.43E-06	6.86E-07	3.43E-06
p, p' DDE	B2	0.34	6.32E-06	4.83E-06	6.31E-06	4.86E-06

[a] USEPA classification: A=known human carcinogen; B2=probable human carcinogen; C= possible human carcinogen; [b]Cancer Potency Factor (mgkg^{-1}bw per d) as per USEPA; P95= 95th percentile

DISCUSSION

In the comparative results of the pesticide groups detected in milk samples, it was observed that OP residues contributed a greater proportion (35.4 %) followed by SPs (32.2 %) and OCs (31.2%) in contradiction to 35% OCs, 28.6% OPs and 27.8% SPs earlier reported [10]. An analysis of results of previous works on pesticide residues in Punjab from 1999 to the present study shows a clear pattern of rapidly declining mean residue levels of HCH and DDT reflecting the shift to the use of OPs and OCs (Figures 1 and 2).

In a survey of pesticide residues in milk samples collected in 2001 in Punjab [10], it was observed that HI for all the pesticides detected except DDT and HCH fell below the value of one. Also, HI for DDT and HCH residues in milk samples from Mumbai, India, were below [18].

The cancer risk for children from lindane is identical with the one for adults for the same pesticide. Similarly, the cancer risks for adult from p,p' DDD and p,p DDE in the LB scenario were 6.79 x 10^{-6} and 6.32 x 10^{-6} respectively while the risks are 3.4 x 10^{-5} and 4.83 x 10^{-6} at the UB level. The cancer risks for children from p,p' DDD and p,p DDE were also found to be within the safe limits. Bedi *et al.* [10] reported similarly low cancer risk levels due to exposure to carcinogenic pesticides as a result of milk consumption in Punjab. The levels of contamination of milk samples with pesticides were found to be minimal in terms of both concentration and frequency of occurrence. Only three samples out of 202 contained DDT and HCH residues, and this is a reflection of the impact of the restriction on the use of these two pesticides in India. Presence of several less-persistent organophosphates and synthetic pyrethroids in milk in this study, even though at generally low levels and frequencies, mirrors the shift to the use of OPs and SPs. Lower levels and frequencies of occurrence found in this study compared to previous one from Punjab and other parts of India might be indications of increasing awareness of and adoption of better pasture and farm management practices among farmers in Punjab. Potential risks of exposure evaluated for both cancer and non-cancer effects based on the estimated dietary intake of pesticide residues revealed that HI for all pesticides were far below 1 for both the lower-bound and upper bound scenarios, reflecting significant improvement over the last two decades. Continuous monitoring of pesticides residues in milk and milk products should be carried out to generate data that will support policies and interventions geared at promoting public health, good management practices, and mitigating public health risks.

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REFERENCES

1. Kannan K, Tanabe S, Ramesh A, Subramanian A, Tatsukawa R. 1992. Persistent organochlorine residues in food stuffs from India and their implications on human dietary exposure. J. Agric. Foo. Chem. 40: 518–524.

2. Peres F, Moreire CJ, Rodrigues M K, Claudio L. 2006. Risk perception and communication regarding pesticide use in rural work: a case study in Rio de Janeiro State Brazil. Int. J. Occup. Environment. Heal. 12: 400–407.

3. Mostafalou, S A, Abdollahi M. 2013. Pesticides and human chronic diseases: evidence mechanisms and perspectives. Toxicol. Appl. Pharmacol. 268: 157-177.

4. Aroniadou-Anderjaska V, Apland JP, Figueiredo TH, Furtado MDA,. Braga M F. 2020. Acetylcholinesterase inhibitors (nerve agent) as weapons of mass destructions: History mechanisms of action and medical countermeasures. Neuropharmacolo. 181: 108298.

5. Zhang Y, Zhang W, Li J, Pang S, Mishra S, Bhatt P, Zeng D, Chen S. 2021. Emerging technologies for degradation of dichlorvos: A review. Int. J. Environment. Res. Publ. Heal. 18(11): 5789.

6. Okereafor S, Garba S, Okunola OJ, Adamu HI. 2022. Organochlorine and organophosphorus pesticide residues in Grains vegetables and fruits. FUDMA J. Sci. 6(4): 174-180.

7. Goulart S, De Queiroz M, Neves AA, De Queiros JH. 2008. Low temperature clean-up method for the determination of pyrethroids in milk using gas chromatography with electron capture detection. Talanta 75: 1320–1323.

8. John PJ, Bakore N, Bhatnagar P. 2001. Assessment of organochlorine pesticide residue levels in dairy milk and buffalo milk from Jaipur city Rajasthan. India. Environment. Int. 126(40): 231-36.

9. Battu RS, Singh B, Kang BK Chahal KK. 2001. Status of contamination of bovine milk with pesticide residues. Abst.PEF. Indian Agricultural Research Institute Nov. 19-23 2001.

10. Bedi JS, Gill JPS, Aulakh RS, Prabhjit K. 2015. Pesticide Residues in Bovine Milk in Punjab India: Spatial Variation and Risk Assessment to Human Health. Arch. Environment. Contaminat. Toxicolo. 69(2): 230-40.

11. Sharma S, Sharma V, Jain JJ, Agarwak S, Dahiya DK, Khan A. 2016. Organochlorine organophosphate synthetic pyrethroids assessment in bovine milk and water samples of Jaipur Rajasthan Indian. J. Ani. Sci. 86(3): 288–293.

12. Battu RS. Singh B, Kang BK. 2004. Contamination of liquid milk and butter with pesticide residues in the Ludhiana district of Punjab state India. Ecotoxicolo. Environment. Safet. 59(3): 324-31.

13. Food and Agriculture Organization and World Health Organization 1997. Guidelines for Predicting Dietary Intake of Pesticide Residues (revised). Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) in collaboration with the Codex Committee on Pesticide Residues WHO/FSF/

FOS/97.7. Rome Italy. http:// who.int.

14. Government of India 2010. Ministry of statistics and programme implementation New Delhi. www.indiastat.com.

15. Food and Agriculture Organization and World Health Organization 2009. Pesticide residues in food. Joint FAO/WHO Meeting on Pesticide Residues.Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues.World Health Organization Food and Agriculture Organization of the United Nations Rome.

16. United States Environmental Protection Agency 2009. www.epa.gov.

17. Dougherty CP, Henricks H, Reinert JC, Panyacosit L, Axelrad DA, Woodruff TJ. 2000. Dietary exposures to food contaminants across the United States. Environment. Res. 84: 170-85.

18. Pandit GG, Sahu SK, 2002. Assessment of risk to public health posed by persistent organochlorine pesticide residues in milk and milk products in Mumbai India. J Environ. Monit. 4: 182–185.