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Carcinogenic Pesticides Residue Detection in Cow Milk and Water Samples from Patna, India

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Abstract

In recent decades, carcinogenic chemicals related to cancer incidence were increased in the industrialized developing world. The present study was carried out to evaluate the selected carcinogenic pesticides (aldrin, chlorpyriphos, DDT, endosulfan, malathion and monocrotophos) residue in cow milk and water samples of Patna, India. Samples were collected from different blocks of Patna and pesticides residue was analyzed by HPLC. Among the all pesticides residue, DDT and endosulfan was found as maximum in cow milk and water samples. High concentrations of DDT (maximum 22 ppb in cow milk and 13 ppb in water samples) and endosulfan (maximum 48 ppb in cow milk and 49 ppb in water samples) were observed in the all samples collected from different blocks of Patna district. Chlorpyriphos and malathion pesticides residue were found below the WHO permissible limit in comparison to the other pesticides in tested cow milk and water samples. This study determined that the presence of carcinogenic pesticides in cow milk and water may increase cancer risk to the people of Patna.

Keywords: HPLC, carcinogen, pesticide, organochlorine, organophosphate

Introduction

Pesticides are extensively used for control of agricultural pests and play an important role in Indian agriculture to meet increasing demands for food and fibre. Since 1950, global pesticide usage was increased 50 fold with the drastic growth of human population [1]. India is the 12th largest producer of chemical pesticides in the world. In India, 217 pesticides are registered under section 9(3) of the Insecticide Act 1968 for use and 65 technical grade pesticides are manufactured indigenously [2]. The average per hectare usage of pesticides in India has gone up from 15.4 g/ha in 1960-61 to 282 g/ha in 2003 and consumption of pesticides is increasing at the rate of 2 to 5% per year [3]. It has been estimated that hardly 0.1% of the agrochemicals used in crop protection reach the target pests and the remaining 99.9% enter the environment [4]. Indiscriminate use of chemical pesticides over the years has adversely affected human health, non-target organisms and environment. Due to higher dose and repeated frequency of application, every year one million people suffer from pesticide poisoning [4]. Jeyaratnam [5] estimated 25 million occupational pesticides poisoning each year among agricultural workers in developing countries alone. The WHO estimated that 849,000 people die globally from self-harm each year [6].

Generally, all pesticides are toxic substances designed to kill pests by their poisonous or deleterious effects. Directly or indirectly pesticides may entered into our body through food chain and ultimately causing various human health impacts allergic reactions to dreadful effects including sterility and cancer. Organochlorines and organophosphates constitute the major synthetic pesticides used for pest control. Among the synthetic pesticides, aldrin, chlorpyriphos, DDT (dichlorodiphenyltrichloroethane), endosulfan, malathion and monocrotophos are much concern because of their toxicity, persistence, accumulation tendency in animals and carcinogenicity in India. Aldrin and monocrotophos are categorized as highly hazardous pesticide (Class Ib), chloropyrifos and endosulfan was classified in moderately hazardous pesticide (Class II), DDT and malathion was categorized as slightly hazardous pesticides [2]. These pesticide chemicals are toxic in high levels and carcinogenic in animal tests. Epidemiological

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studies indicate that some of the synthetic pesticides are acting as endocrine disturbing chemicals and causing several hormone-related cancers.

Diamanti-Kandarakis et al. [7] and Fleseriu [8] reported that aldrin, chloropyrifos, DDT, endosulfan, malathion and monocrotophos pesticides are acting as endocrine disturbing chemicals on endocrine system of animals and may cause cancer risks. There is a possible association between human exposure to the pesticide chemicals and hormone-dependent human cancers on reproductive tissues of breast, testis, prostate, ovary and endometrium [9]. According to International Agency for Research on Cancer, aldrin is considered as Group A3 carcinogen [10]. Alavanja et al. [11] reported that chlorpyriphos exposure increases prostate cancer rates in men. DDT is a well known possible human carcinogen (Group 2B) and it may increase breast cancer risk [12]. Nath et al. [13] studied the carcinogenic effect of endosulfan on mice and positive result was recorded in ovary. Endosulfan may increase breast cancer risk comparable to that of DDT [14]. Malathion and monocrotophos are highly toxic chemicals, but carcinogenic evidence data was very less. While, with the report of Cox [15] and Watts [16], it is considered that malathion and monocrotophos has been linked with liver and breast cancer risks respectively.

Increase in human population growth rate demands of more food and energy, indirectly resulting in high consumption of pesticides and contamination in environment. Exposure to agricultural pesticide is mostly through contaminated food and drinking water. Pesticides can also reach ground water resources [17]. India started using pesticides in the year 1948 and pesticide production in the year 1952 [18]. Today synthetic carcinogenic pesticide chemicals contamination is pervasive everywhere. Most organochlorine and organophosphorous pesticides are more stable in the environment. The data available from a large number of countries showed that most frequently used highly toxic pesticides have become widely contaminated in water and cow milk. Previously, many authors were reported that the presence of pesticide residues in water and food sources from different parts and states of India. Though, in the sate of Bihar, pesticide contamination data was not available in water and cow milk. The present study has been aimed to monitor the contaminated level of most important carcinogenic pesticide residues (aldrin, chloropyrifos, DDT, endosulfan, malathion and monocrotophos) in raw cow milk and water samples of Patna, India.

Materials and Methods

Chemicals

Pesticide standard fine organochlorine (aldrin, DDT and endosulfan) and organophosphate (chloropyrifos, malathion and monocrotophos) chemicals were obtained from Sigma-Aldrich, India. Purity of the obtained chemicals was 99%. The standard solutions of pesticides were prepared in n-hexane. HPLC grade solvents and all other chemicals (analytical grade) were purchased from Himedia Chemicals Pvt. Ltd., India.

Sampling and collection

Twenty blocks were selected from the Patna district of Bihar state, India for sampling. In each block, samples were collected from three different sites near to agricultural activity and used as replicates. This was done to assess the contamination level in cow milk and water samples. Five hundred milliliter of land water and fresh cow milk samples were collected directly from selected sites and milkmaids respectively on the period from March 2012 to May 2012.

HPLC analysis

All the collected samples were immediately brought in to the chromatography laboratory at the Research Centre of Mahavir Cancer Sansthan, Patna. Pesticide extraction, purification and residual analysis were done according to a method described by Sosan et al. [19] for water sample and Ashnagar et al. [20] for milk sample analysis. Purified pesticide extracts were made up to an appropriate volume and analysed by HPLC (High Performance Liquid Chromatography) after which the retention times of organochorine and organophosphate chemical residues and standards were compared. HPLC (PerkinElmer Series 200) instrument equipped with a UV detector and auto-sampler was used. The optimum HPLC chromatography was showed in Table 1.

Results

The detection limits for the tested organochorine and organophosphate pesticide chemicals are presented in Table 2. On the basis of these standard pesticides considerations, pesticide extracts from the collected cow milk and water samples were tested in the HPLC instrument. Isomers of DDT (ppDDT, ppDDE and ppDDD) and endosulfan (α-endosulfan and β-endosulfan) pesticides were investigated with aldrin, chlorpyriphos, malathion and monocrotophos pesticides. Finally, the total DDT and endosulfan residual levels in the cow milk and water samples were quantified from the DDT and endosulfan isomers. The results expressed on residual level in percentage of all pesticides in cow milk

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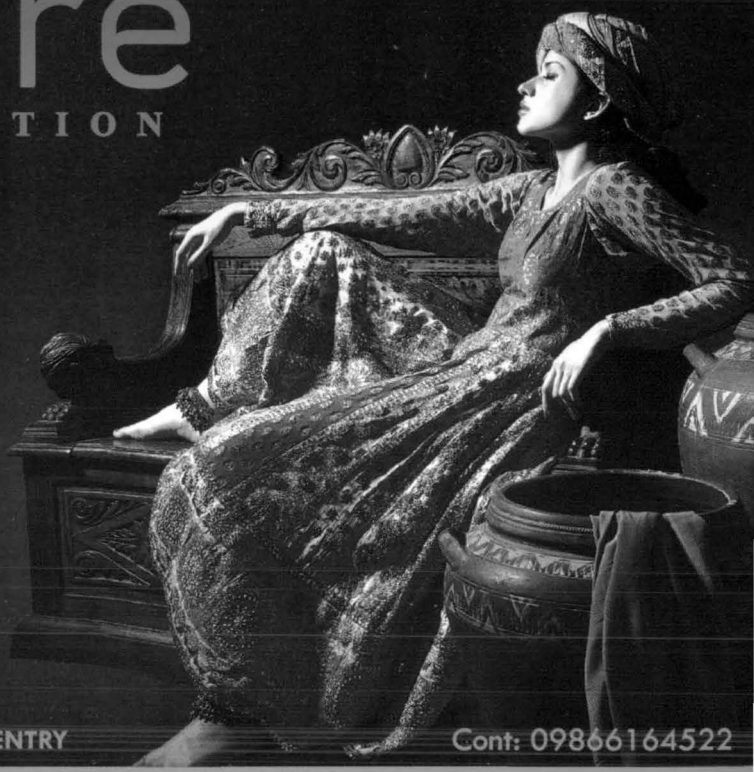

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and water samples from Patna district are presented in Table 3. Residual level of DDT and endosulfan was found as maximum among the all pesticides in cow milk and water samples. In cow milk samples, DDT was detected in 38% and endosulfan was detected in 33% of all the samples analysed. DDT was detected in 55% and endosulfan was detected in 30% of all the water samples analysed. Aldrin and monocrotophos was not detected in cow milk samples, and chlorpyriphos and monocrotophos was not detected in water samples.

Of the 23 blocks of Patna, cow milk and water samples were collected from the different sites of 20 blocks (Athmalgola, Bakhtiyarpur, Barh, Bihta, Bikram, Danapur, Daniyawan, Dhanarua, Dulhin Bajar, Fatuha Circle, Ghoswari, Mokama, Naubatpur, Paliganj, Pandarakh, Patna Sadar, Phulwarisharif and Punpun) and it was performed in HPLC. Table 4 showed the results of pesticides residue detection in cow milk and water samples of different blocks of Patna district. Among the 20 blocks, pesticides residue was not detected in the Belchhi, Khusrpur, Pandarakh and Phulwarisharif blocks. DDT and endosulfan was found in the most of the cow milk and water samples followed by malathion. Maximum residual level of DDT in cow milk (22.2 ppb) and water (13.5 ppb) was recorded in Bihta and Naubatpur blocks respectively. In cow milk, 48.6 ppb level of endosulfan was recorded as maximum in Patna Sadar, and in water, 49.4 ppb level of endosulfan was recorded as maximum in Danapur. Maximum 27.7 ppb concentration of malathion was found in cow milk sample at Athmalgola block and 14.6 ppb concentration of malathion was found in water sample at Bikram.

Table 1. HPLC conditions for pesticide residue analysis.

HPLC Conditions		
Column	:	Silica gel pak C18 (5µm, 250x4.6mm)
Mobile phase	:	Methanol : Water (9:1)
Flow rate	:	1 ml/min
Temperature	:	Ambient
UV Detection	:	214 nm
Injector volume	:	15 µl
Standard Conc.	:	500 µg/ml

Chlorpyriphos (1.6 ppb) was only found in cow milk sample of Dhanarua and aldrin (0.26 ppb) was only found in water sample of Patna Sadar.

The results of this study revealed that the pesticides contamination in cow milk and water of Patna and the compiled data was presented in Table 5. When compared to WHO permissible limit in water, aldrin, DDT and endosulfan contamination was higher in water samples. Similarly, in cow milk also chlorpyriphos, DDT and endosulfan contamination level was higher. Among the analysed pesticides, malathion level was under permissible limit and monocrotophos level was not

Table 2. Retention time and peak area of standard pesticides in HPLC performance

Pesticide	Retention Time (min)	Peak Area (m^2)
Aldrin	10.93	565275
α-endosulfan	3.88	341118
β-endosulfan	5.87	346834
Chlorpyriphos	5.07	2288191
ppDDD	6.45	2207677
ppDDE	9.85	2642547
ppDDT	8.06	897466
Malathion	3.20	1413850
Monocrotophos	2.58	1776167

Table 3. Percentage of pesticide residues detection in total number of cow milk and water samples of Patna, India

Pesticide residues	% Contamination in cow milk samples	% Contamination in water samples
Aldrin	0.00	5.00
Chlorpyriphos	5.56	0.00
Total DDT	38.89	55.00
Total Endosulfan	33.33	30.00
Malathion	22.22	10.00
Monocrotophos	0.00	0.00

Table 4. Mean level of pesticide residues in cow milk and water samples of different blocks of Patna, India

Sampling site	Pesticide residues in cow milk sample (ppb)						Pesticide residues in water sample (ppb)					
	Al	Ch	Di	En	Ma	Mo	Al	Ch	Di	En	Ma	Mo
Athmalgola	-	-	-	-	27.7	-	-	-	5.1	-	-	-
Bahtiyarpur	-	-	-	-	22.0	-	-	-	4.0	-	-	-
Barh	-	-	-	13.0	-	-	-	-	-	37.7	-	-
Belchhi	-	-	-	-	-	-	-	-	-	-	-	-
Bihta	-	-	22.2	-	-	-	-	-	-	-	-	-
Bikram	-	-	11.0	37.3	-	-	-	-	-	2.8	14.6	-
Danapur	-	-	2.6	41.9	-	-	-	-	5.5	49.4	-	-
Daniyawan	-	-	-	-	16.9	-	-	-	3.9	-	-	-
Dhanarua	-	1.6	7.3	-	-	-	-	-	-	27.1	-	-
Dulhin Bajar	-	-	12.1	-	-	-	-	-	3.6	-	-	-
Fatuha Circle	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
Ghoswari	-	-	6.9	-	-	-	-	-	-	32.7	-	-
Khusrupur	-	-	-	-	-	-	-	-	-	-	-	-
Maner	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
Masaurhi	-	-	-	-	-	-	-	-	4.2	-	-	-
Mokama	-	-	-	-	14.4	-	-	-	3.3	-	-	-
Naubatpur	-	-	6.3	18.5	-	-	-	-	13.5	-	-	-
Paliganj	-	-	-	23.9	-	-	-	-	4.2	-	2.3	-
Pandarakh	-	-	-	-	-	-	-	-	-	-	-	-
Patna Sadar	-	-	5.7	48.6	-	-	0.26	-	13.0	47.7	-	-
Phulwarisharif	-	-	-	-	-	-	-	-	-	-	-	-
Punpun	-	-	-	-	-	-	-	-	-	40.7	-	-
Sampatchak	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt

Al – Aldrin; Ch – Chlorpyriphos; Di – Dichlorodiphenyltrichloroethane; En – Endosulfan; Ma – Malathion; Mo - Monocrotophos

Table 5. Contamination of pesticides residue in cow milk and water samples of Patna, India

Sampling Site and Sample		Residual level of pesticide in ppb (Mean ± SD)					
		Al	Ch	Di	En	Ma	Mo
Patna	Cow Milk	-	1.60	9.26 ± 6.02	30.53 ± 14.13	20.20 ± 5.89	
district	Water	0.26± 0.00	-	6.03 ± 3.86	34.01 ± 15.84	8.45 ± 8.69	-
WHO/EPA standard limit in water (Hamilton et al. 2003)		0.03	20.00	2.00	0.05	100.00	1.00

Al – Aldrin; Ch – Chlorpyriphos; Di – Dichlorodiphenyltrichloroethane;
En – Endosulfan; Ma – Malathion; Mo - Monocrotophos

Discussion

India is a rapidly developing and agrarian country. Country need to increase food production to compensate human needs for rapidly growing population is well recognized. Nearly, 45% of annual food production is lost due to pest infestation [21]. In general, chemical pesticides plays major role to protect crops from pests and diseases. In view of that, chemical pesticide consumption was increased in the country. On the other hand, synthetic carcinogens and endocrine disturbing chemicals including pesticides related cancer incidence was also increased during last 50 years [7]. Indian Council of Medical Research (ICMR) was also reported that cancer incidence and endocrine disorders are increasing in India due to chronic pesticide poisoning [22]. On the basis of these importances, the present work was primarily attempted to evaluate carcinogenic pesticides contamination in cow milk and water sources from human dwelling areas of Patna, India.

Among the chosen carcinogenic pesticides in the present study, aldrin was banned, DDT was restricted in use, and the other pesticides are in wide use in India [2]. Recently, India witnessed a furor over banning endosulfan, with reports appearing on endosulfan poisoning [23]. The results revealed that aldrin and monocrotophos was not detected and the remaining pesticides chlorpyriphos, DDT, endosulfan and malathion were detected in cow milk and water sources by HPLC analysis. Of the detected carcinogenic pesticide chemicals, DDT and endosulfan was found as most prominent contaminants in cow milk and water of the studied Patna region. Kumar et al. [24] reported that DDT was sprayed frequently (frequent years) in the different districts of Bihar for vector control programme especially for kala-azar disease. Recently, DDT was sprayed in the year of 2007 in Bihar [24]. Endosulfan was widely used to control economically important some agricultural insect pests in Bihar as well as all over the country, which was recently banned in Bihar [23]. Consequently, both DDT and endosulfan may be contaminated in the studied region and it was detected in cow milk and water samples.

In general, organochlorines are highly stable in nature, persist for long time and tend to accumulate in the environment, because of low solubility in water and high stability in sunlight. Likely, DDT and endosulfan has low solubility in water, high lipid solubility and stability in fatty tissue. According to Wrigley [25], fat soluble DDT and endosulfan might be absorbed into the leaves of certain edible plants and it may enter into cow milk. IPCS [26] report denoted that over 90% of the DDT stored in the general population is derived from food sources including milk and water. In our previous study, we found that high level of residual accumulation of DDT and its metabolites in the blood (5 to 25 ppb) and tissue (900 - 4300 ppb) of breast cancer patients and also it was recorded that breast cancer incidence was increased during the period of 2000 to 2009 in the state of Bihar, India [27]. Djordjevic et al. [28] and Wolff et al. [29] reported that the association of breast cancer risk and endosulfan levels in blood and

tissue of women. In the point of view, present and earlier study results may correlate that contamination of carcinogenic DDT and endosulfan in water, cow milk and blood in the breast cancer risk patients of Bihar.

Followed by organochlorine pesticides, organophosphates are the most widely used group of in the world. Among the different types of synthetic pesticides used, nearly 30% of pesticides are belongs to organophosphorous group in India [30]. Organophosphorous are easily soluble in water and most toxic to insect pests, animals and human. In the present study, it was observed that chlorpyriphos was detected in cow milk, and malathion was detected in both cow milk and water. Both chlorpyriphos and malathion pesticides are commonly used to control agricultural insect pests in Bihar. In addition, malathion was widely used for vector control programmes especially for malaria and kala-azar diseases. When compared to chlorpyriphos, malathion was highly accumulated in both cow milk and water samples. Recently, Jean et al. [31] found the most frequent contamination of malathion than the chlorpyriphos residues in food samples. Ahuja [32] has detected monocrotophos residues over the prescribed maximum residue limit in cauliflower, cabbage, tomatoes, brinjal, okras, french beans and cucumbers and Ali [33] has reported chlorpyriphos level in water.

Conclusion

When compared to WHO/EPA permissible limit, DDT and endosulfan are highly contaminated in the tested cow milk and water samples of Patna. Aldrin was also recorded over the permissible limit in water. Chlorpyriphos and malathion were found below the permissible limit.

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