ASSESSMENT OF LINDANE (GAMMA HCH) RESIDUES IN WATER AND MILK SAMPLES IN PALAKKAD DISTRICT*

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Abstract

Organochlorine pesticides are ubiquitous and persistent organic pollutants used widely throughout the world. The organochlorines are more potent due to their prolonged persistence, lipophilic nature and tendency to accumulate in animal and plant tissues. The present study was therefore undertaken to assess the level of lindane residues in water bodies and milk samples from cattle of Pudussery and Perumatty panchayath of Palakkad district, Kerala. Total mean lindane (Gamma HCH) levels in the water and milk samples (ppm) from study area were $1.935 \times 10^{-4} \pm 6.779 \times 10^{-5}$ and $2.600 \times 10^{-3} \pm 7.734 \times 10^{-4}$ respectively. The levels of lindane residues (ppm) in water and milk samples were within the maximum residue limit as per FAO/ WHO recommendations.

Keywords: *Lindane, Gas chromatography*

Organochlorine pesticides are ubiquitous environmental contaminants that are very stable and remain in the environment for long periods of time. Many organochlorine pesticides are persistent organic pollutants because of their prolonged persistence, lipophilic nature and tendency to accumulate in animal and plant tissues. Although banned or restricted in most of the developed countries, some of the developing countries are still using the organochlorine pesticides, being less costly and more effective in controlling ectoparasites. Lindane and endosulfan are currently used organochlorine pesticides with numerous documented negative health impacts. Lindane is a pesticide used as an agricultural insecticide and also as an acaricide in animals. In May 2009, governments from countries around the world included lindane to be phased out under provisions of the Stockholm Convention on Persistent Organic Pollutants (POPs), a global United Nations (UN) treaty to eliminate the use of POPs (UNEP, 2009).

Materials and Methods

Sample collection

Water samples and milk samples (20 each) were collected as per the method described by Lorgue *et al.* (1996). Water samples were collected from ponds, wells, paddy fields and marshy areas of study area in clean sampling bottles. Milk samples were

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collected from 20 adult cattle which were maintained for a period of three years in the study area. All the samples collected were stored under deep freezing (-20°C) conditions.

Residue Extraction

From the collected water samples, five hundred milliliter water was taken for analysis and residue extraction was done as per method prescribed by Sherma, (1979). In case of milk samples, about 100ml was taken for analysis and residue was extracted in 2 ml n-hexane for injection into gas chromatograph. (FDA ,1977)

Lindane content in water and milk samples were quantified by Gas chromatography. Analysis was done in SHIMADZU-2010 A with electron capture detector (ECD) having 63 Ni as the radioactive source.

Calibration of instrument

Standard solutions of HCH (alpha, beta, delta and gamma) were procured from Sigma- Aldrich. Stock solution of 1 ppm was prepared for each isomer using n-hexane as solvent. The ECD response for all the isomers were analysed individually at a range of concentrations from 0.01ppm to 0.5 ppm. Linearity was observed for all isomers up to a concentration of 0.5 ppm.Then a multilevel calibration was made using four different concentrations (0.01, 0.025, 0.05 and 0.5ppm**).** The chromatogram of standard is shown in Fig. 1.

Detection and estimation

The chromatogram of samples and pesticide standard were obtained under identical operation conditions of Gas liquid chromatography. The residues in unknown sample were detected by the comparison of their retention time with the standard. Sum total of pesticide residue and its isomers were quantified by the formula

Pesticide residue in ppm = Amount obtained x V/M

V = Total volume of cleaned up sample in ml

M = Weight (g) or Volume (ml) of sample taken for extraction.

Results and Discussion

Total mean lindane(Gamma HCH) levels in the water and milk samples (ppm) from study area were $1.935 \times 10^{-4} \pm 6.779 \times 10^{-5}$ and $2.600 \times 10^{-3} \pm 7.734 \times 10^{-4}$ respectively. The Lindane levels in water and milk samples are presented in Table 1. The chromatogram of the water and milk samples from the study area is shown in Figs.2 & 3.

Table 1. Gamma HCH (Lindane) levels in water and milk samples from Pudussery and Perumatty area

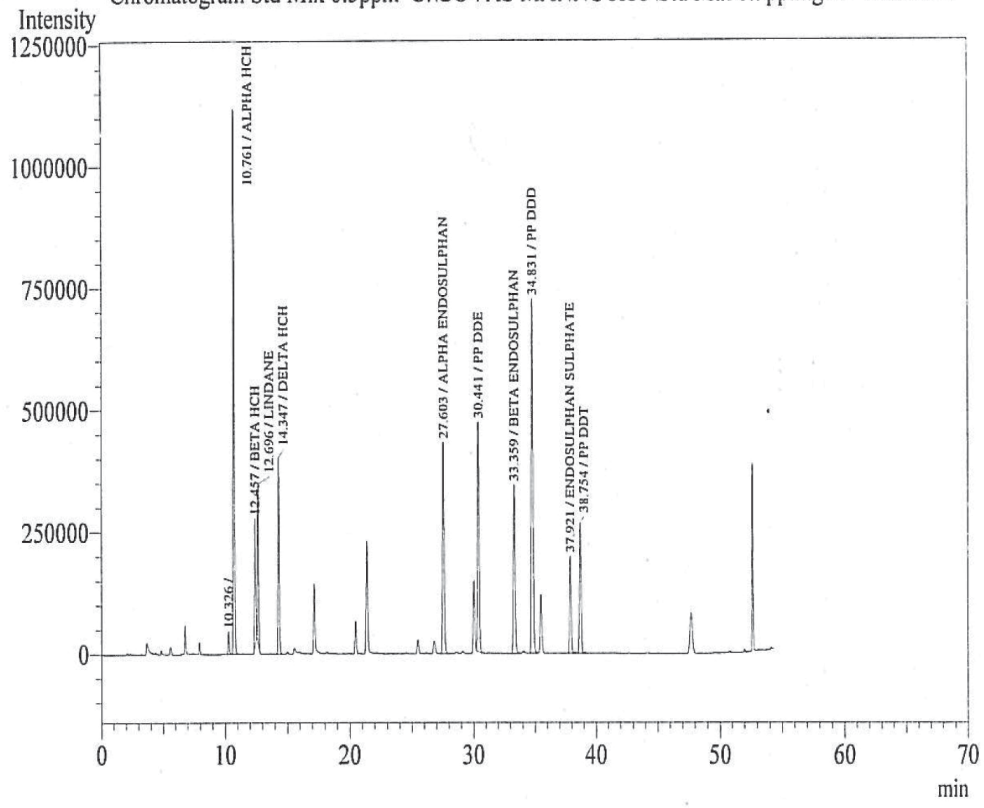
Sample No	GammaHCH(ppm)	GammaHCH(ppm)
1	0.00045	0.002
2	0.0003	0.0027
3	0.00007	0.0012
4	0.00007	0.001
5	0.00009	0.0007
6	0.00003	0.012
7	0.00001	0.003
8	ND	0.0008
9	0.00043	0.003
10	0.00095	ND
11	0.00054	0.0022
12	0.00005	0.0027
13	ND	0.0021
14	0.00002	0.00001
15	0.00002	0.0011
16	0.00001	0.00074
17	0.00091	0.0126
18	ND	0.00205
19	ND	ND
20	ND	0.002
Mean± SE	$1.93 \times 10^{-4} \pm 6.779 \times 10^{-5}$	$2.600 \times 10^{-3} \pm 7.734 \times 10^{-4}$

According to the European Economic Commission (EEC Directive 80/778/EEC) the total pesticide level in drinking water should not exceed 0.5 ìg/l and an individual pesticide not greater than 0.1 ìg/l. The results of this study are in agreement with study conducted by Zhang *et al*. (2002) in which they reported that the level of organochlorine pesticide in Wuchan river in China were below the guideline value. Lindane enters water from direct application for the control of mosquitoes, from use in agriculture and forestry and to a lesser extent, from occasional contamination of waste water from manufacturing plants. The persistent nature of organochlorines and their translocation from the agricultural land as runoff or as irrigation return flow can cause residues in water sources. These pesticides eventually end up in ground water and their

Sample Information

nalysis Date & Time: 5/19/2011 10:27:05 AM
ser Name : Admin
ımple Name : Std Mix 0.5ppm
ımple ID : Std Mix 0.5ppm
ımple Type : Unknown
jection Volume : 2.00
ata Name : C:\COVAS MANNUTHY\Std Mix 0.5ppm.gcd
ethod Name : C:\GC 3\2011\April 2011\22 OC,OP& SP Mix CALI (0.01-0.5PPM).gcm
eport Name : C:\WINDOWS\System\DEFAULT.gcr
atch Name : C:\COVAS MANNUTHY\MILK SAMPLES-19-05-11.gcb

Chromatogram Std Mix 0.5ppm C:\COVAS MANNUTHY\Std Mix 0.5ppm.gcd - Channel 1



Quantitative Results - Channel 1

ID#	Name	Ret.Time	Area	Height	Conc.	Units
1	ALPHA HCH	10.761	6787898	1116376	0.000	ppm
2	BETA HCH	12.457	1674127	257282	0.000	ppm
3	LINDANE	12.696	1904426	327290	0.000	ppm
4	DELTA HCH	14.347	2750217	403912	0.000	ppm
5	ALPHA ENDOSULPHAN	27.603	3949596	433513	0.000	ppm
6	PP DDE	30.441	4106643	469737	0.000	ppm
7	BETA ENDOSULPHAN	33.359	3289507	345140	0.000	ppm
8	PP DDD	34.831	6727034	725326	0.000	ppm
9	ENDOSULPHAN SULPHATE	37.921	1845793	198689	0.000	ppm
10	PP DDT	38.754	2447549	266996	0.000	ppm

Fig. 1. Chromatogram of standard and their retention times

Sample Information

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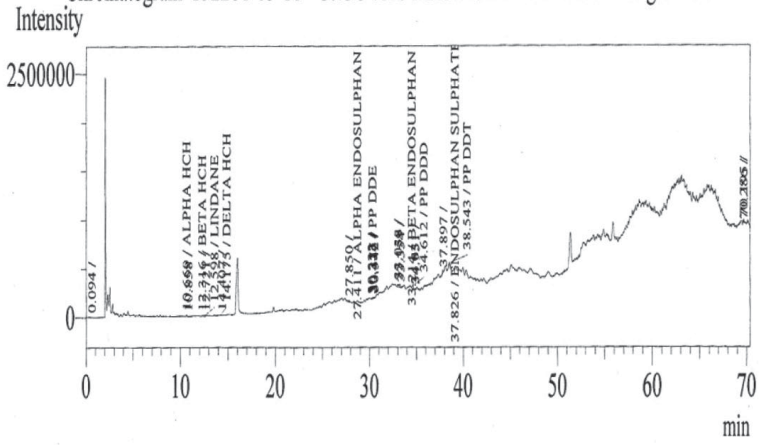
Sample Name : R 2261-05-11
Sample ID : R 2261-05-11
Sample Type : Unknown
Injection Volume : 2.00
Data Name : C:\COVAS MANNUTHY\R 2261-05-11.gcd
Method Name : C:\GC 3\2011\April 2011\22 OC,OP& SP Mix CALI (0.01-0.5PPM).gcm
Report Name : C:\WINDOWS\System\DEFAULT.gcr
Batch Name : C:\COVAS MANNUTHY\MILK SAMPLES.gcb

Chromatogram R 2261-05-11 C:\COVAS MANNUTHY\R 2261-05-11.gcd - Channel 1



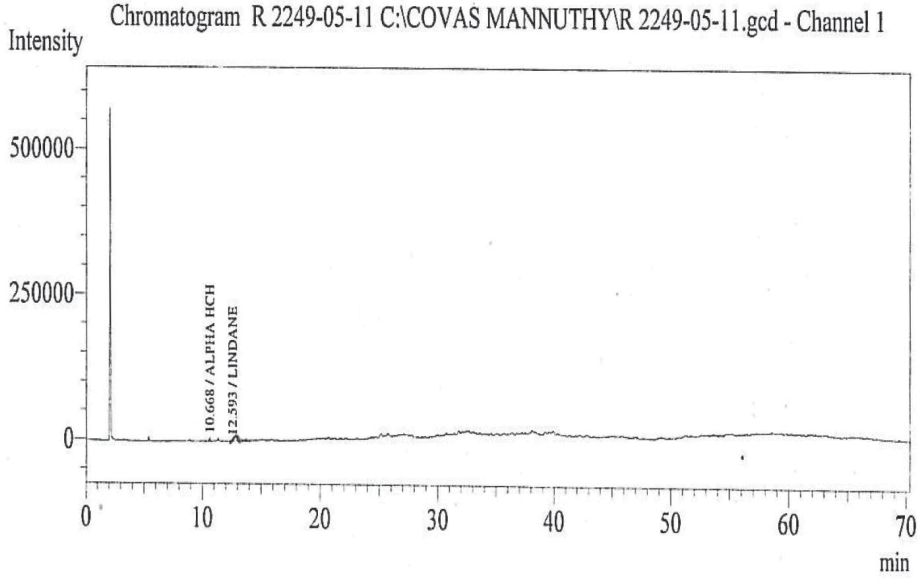
Quantitative Results - Channel 1

ID#	Name	Ret.Time	Area	Height	Conc.	Units
1	PHORATE	0.000	0	0	0.000	ppm
2	ALPHA HCH	10.669	93973	14237	0.013	ppm
3	BETA HCH	12.316	11275	2026	-0.009	ppm
4	LINDANE	12.598	49188	7896	0.037	ppm
5	DELTA HCH	14.175	17675	2433	0.011	ppm
6	METHYL PARATHION	0.000	0	0	0.000	ppm
7	MALATHION	0.000	0	0	0.000	ppm
8	CHLORPYRIFOS	0.000	0	0	0.000	ppm
9	QUINALPHOS	0.000	0	0	0.000	ppm
10	ALPHA ENDOSULPHAN	27.411	32124	6402	-0.002	ppm
11	PROFENOPHOS	0.000	0	0	0.000	ppm
12	PP DDE	30.313	22886	5861	0.000	ppm
13	BETA ENDOSULPHAN	33.214	192116	31762	0.048	ppm
14	PP DDD	34.612	258976	38049	0.026	ppm
15	ETHION	0.000	0	0	0.000	ppm
16	ENDOSULPHAN SULPHATE	37.826	205008	38381	0.141	ppm
17	PP DDT	38.543	249627	33698	0.731	ppm

Fig.2. Chromatogram of water sample

Sample Information

Analysis Date & Time: 5/18/2011 5:28:51 PM
User Name : Admin
Sample Name : R 2249-05-11
Sample ID : R 2249-05-11
Sample Type : Unknown
Injection Volume : 2.00
Data Name : C:\COVAS MANNUTHY\R 2249-05-11.gcd
Method Name : C:\GC 3\2011\April 2011\22 OC,OP& SP Mix CALI (0.01-0.5PPM).gcm
Report Name : C:\WINDOWS\System\DEFAULT.gcr
Batch Name : C:\COVAS MANNUTHY\MILK SAMPLES.gcb



Quantitative Results - Channel 1

ID#	Name	Ret.Time	Area	Height	Conc.	Units
1	PHORATE	0.000	0	0	0.000	ppm
2	ALPHA HCH	10.668	29983	5617	0.003	ppm
3	BETA HCH	0.000	0	0	0.000	ppm
4	LINDANE	12.593	9603	1767	0.011	ppm
5	DELTA HCH	0.000	0	0	0.000	ppm
6	METHYL PARATHION	0.000	0	0	0.000	ppm
7	MALATHION	0.000	0	0	0.000	ppm
8	CHLORPYRIFOS	0.000	0	0	0.000	ppm
9	QUINALPHOS	0.000	0	0	0.000	ppm
10	ALPHA ENDOSULPHAN	0.000	0	0	0.000	ppm
11	PROFENOPHOS	0.000	0	0	0.000	ppm
12	PP DDE	0.000	0	0	0.000	ppm
13	BETA ENDOSULPHAN	0.000	0	0	0.000	ppm
14	PP DDD	0.000	0	0	0.000	ppm
15	ETHION	0.000	0	0	0.000	ppm
16	ENDOSULPHAN SULPHATE	0.000	0	0	0.000	ppm
17	PP DDT	0.000	0	0	0.000	ppm

Fig.3. Chromatogram of milk sample

transformation products remain in soil and water for long time. (Sankararamakrishnan *et al*., 2005).

According to FAO/WHO the MRL of lindane in milk is 0.01ppm. In this study the lindane residues were below MRL value. Similar studies conducted by Ahmed and Zaki (2009) and Pandit *et al.* (2002) detected the residual content of organochlorine in milk samples and found to be below the maximum permissible limit given by FAO/WHO. Physicochemical properties of these toxins, especially their high lipophilicity, facilitate the absorption and storage of these toxins in human and animal bodies. The usage of highly persistent organochlorines has resulted in trace contamination of air, water and soil with the residues. The residues of these pesticides are accumulated in milk producing animals such as buffaloes and cows, if they feed on contaminated hay/grass or inhale contaminated air.

REFERENCES

Ahmed, N.S. and Zaki, E.M.S. 2009. Detection of some organochlorine pesticides in raw milk in Giza Governorate. *J. Appl. Sci. Res.* **5**: 2520-2523

EFSA. 2005a. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to gamma-HCH and other hexachlorocyclohexanes as undesirable substances in animal feed. *The EFSA Journal.* 250: 1 – 39

EEC Council Directive 80/778/EEC. 1976. *Official Journal of European Communities*, vol.L.229; p.11.

FAO/WHO. 2009. *Food standards Programme*. CODEX Alimentarious Pesticide Residues in Food- Maximum Residue Limits.

FDA.1977. *Pesticide Analytical Manual-Vol 1*. National Technical Service, US. Department of Commerce, Section 211, p.418

Lorgue, G., Lechenet, J. and Riviera, A.1996. *Clinical Veterinary Toxicology*. Blackwell Science. Ltd., London, 251p

Pandit, G.G., Sharma, S., Srivastava, P.K.and Sahu, S.K. 2002. Persistent organochlorine pesticide residues in milk and dairy products in India. *Food. Addit. Contam*. **19**: 153-157.

Sankararamakrishnan, N., Sharma, A. K. and Sanghi,R. 2005. Organochlorine and organophosphorus pesticide residues in ground water and surface waters of Kanpur,Uttar Pradesh, India. *Environ. Int*. **31**: 113-120

Sherma, J. 1979.*Manual for analytical quality control for pesticides and related compounds in human and environmental samples*. United States Environmental Research Triangle, Park Nc- 2771, 312p.

UNEP, Press release on Stockholm Convention. 2009.available at www.unep.org. Accessed on 14-03-12.