# LINDANE (γ-HEXACHLOROCYCLOHEXANE) IN VEGETABLES AND HUMAN HEALTH RISK

Kumar B[1], Sajwan K[2*], Mishra M[1], Verma VK[1], Kumar S[1], Sharma CS[1], Akolkar AB[1]

[1]National Reference Trace Organics Laboratory, Central Pollution Control Board, East Arjun Nagar, Delhi, India-110032; [2]Department of Natural Resources, Savannah State University, Savannah, GA 31404, USA

## Introduction

Lindane (γ-Hexachlorocyclohexane) (γ-HCH) is persistent bioaccumulative contaminants that is found ubiquitously in environment and biological matrices[1], and listed by the Stockholm Convention as persistent organic pollutants (POPs)[2]. The use lindane has been banned or severely restricted in several countries, but still is use in some countries including India[3-4].

Few studies on lindane in vegetables have been carried out in India[5-7] but, quantitative health risk from lindane in vegetables of India is not much investigated[8]. Majority of people in India are vegetarian and vegetables are important components of the human diet due to their high nutritional value and fibre contents. The information on toxic contaminants in vegetables is very essential for protection of human health, because the main nonoccupational route of exposure to toxicants is through dietary intake.

This study was undertaken to assess the daily dietary intake of lindane through vegetables and their potential health risks in terms of incremental lifetime cancer risk (ILCR) and non-cancer health hazard index (HI) for human population.

## Materials and Methods

Solvents, Chemicals and Standards

HPLC grade solvents (acetone, methanol, dichloromethane, and hexane) and analytical grade chemicals (sodium sulphate, silver nitrate, potassium hydroxide, activated charcoal and sulphuric acid) were purchased from Merck (Delhi, India). Silica gel 60 (0.063-0.100 mm) was from Sigma-Aldrich, Co. (St. Louis, Mo). Silica gel and granular anhydrous sodium sulphate was cleaned in Soxhlet extractor with methanol, dichloromethane and acetone for 8 h each, then stored in air tight desiccator until use. Pesticide standard solution was purchased from Supelco (Sigma-Aldrich), and the working standard solution was prepared by diluting the standard stock in hexane.

Sample Extraction and Cleanup

Six vegetables, carrot (*Daucus carota* L.), cauliflower (*Brassica oleracea* L.), eggplant (*Solanum melongena* L.), radish (*Raphanus sativus* L.), spinach (*Spinacia oleracea* L.), and tomato (S. *lycopersicum* L.) collected from West Bengal, Eastern India. After washing, vegetables cut into small pieces with a grater and thoroughly mixed. A known quantity of samples were macerated with anhydrous sodium sulphate and extracted three times with acetone on a mechanical shaker. The acetone extract was filtered with vacuum suction and concentrated to near 20-25mL using a rotatory vacuum evaporator (Buchi, Essen, Germany). The concentrated extracts were subjected to liquid-liquid partitioning three times with hexane in a separatory funnel. Hexane layer with residues was collected by passing the sample through sodium sulphate and concentrated to 5-10 mL. Pigment removal was carried out using activated charcoal, and multilayered silica gel column chromatography was performed for removal of interfering compounds and other aliphatic compounds.

Analysis and Quality Control

Content of α-HCH (Lindane) in vegetables were identified and quantified using a gas chromatograph (3400cx, Varian Star, California, USA) equipped with $^{63}$Ni electron capture detector. Sample extracts of 1µL was separated on a capillary column (RTX-5) (30 m x 0.25mm id) coated with a 0.5µm of stationary phase of 5% diphenyl 95% dimethylpolysiloxane. The column oven temperature program was: initially maintained at 170 $^{0}$C and programmed to 220 $^{0}$C (@ 7 $^{0}$C min$^{-1}$), the temperature was increased @ 5 $^{0}$C min$^{-1}$ to 250 $^{0}$C and held for 7.0 min. The injector and detector temperature were 250 and 350 $^{0}$C respectively. Nitrogen gas was used as the carrier (@1.0mL min$^{-1}$).

Certified reference standard solution was used for instrument calibration and other quality control studies. Concentration of γ-HCH was determined with an external standard method comparing peak area in samples with the standards using Varian Star workstation software. Peak identification was conducted with the accurate retention time of each standard using five levels of calibration curves of standards ($r^2$ value, 0.999). Quality assurance/quality control analyses were performed, which include analysis of procedural blanks, duplicate sample analysis (standard deviation <5), calibration verification (standard deviation <5) and matrix spiked study. Samples were spiked with known working standard solutions of pesticides, then extracted and analyzed in the same way as the real samples. Recoveries were assumed to be satisfactory for each pesticide compound. Each sample was analysed in duplicate and the average of representative data used in calculations. Levels below reporting limit (0.01 µg kg$^{-1}$) were considered as zero in calculations.

Health Risk Estimation

Considering the toxicological effects of γ-HCH, it is important to investigate the potential exposures of humans through dietary intakes. γ-HCH intake through vegetables was estimated as lifetime average daily dose (LADD), considering that an adult human (age, 60 year; BW, 65 kg) consume 137 g d$^{-1}$ vegetables on an average for all the days in a year. Incremental lifetime cancer risk (ILCR) and non-cancer health hazard (Hazard Quotient, HQ) to humans was assessed following Risk Assessment Guidance[1, 9-10].

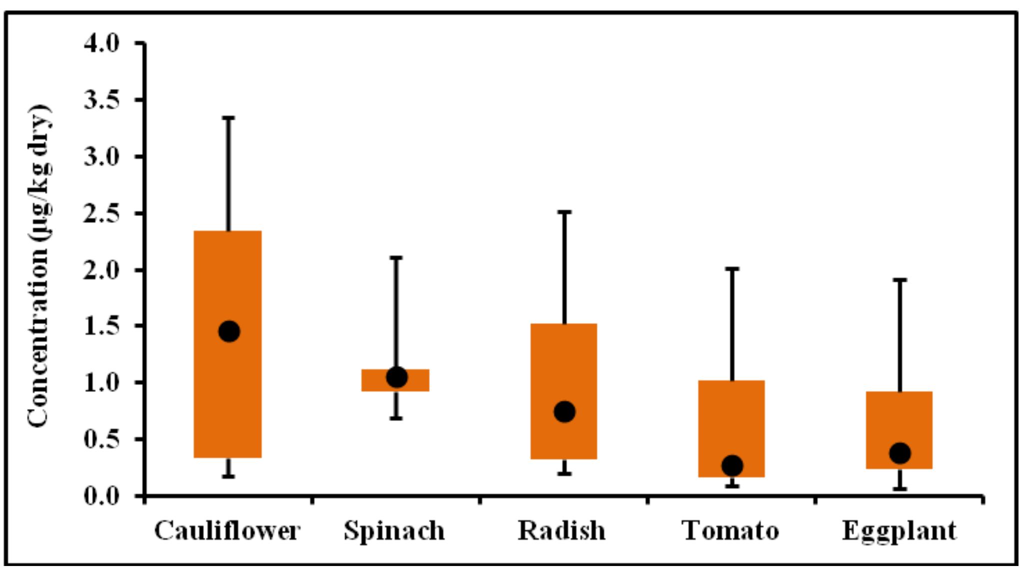


**Fig. 1**: Box and Whisker Plot for the Lindane in selected vegetables

**Results and Discussion**

Concentration means and ranges of Lindane in different vegetables were presented in Figure 1. The observed level of γ-HCH ranged between 0.06-6.61 µg $kg^{-1}$ with a mean of 1.37 µg $kg^{-1}$. The usage of technical HCH and lindane has been banned in agriculture but, used in public health programmes to control vector borne diseases[4]. The observed levels of lindane in vegetables were compared with maximum residual levels (MRLs) and found lower than the recommended values from European countries and Indian government[11-12]. Human health risk due to Lindane through vegetables was assessed, considering that humans take vegetables all the days in a year during the life span. Risk was assessed by estimating the incremental life time average daily dose (LADD) and subsequently their incremental life time cancer risk (ILCR) and non-cancer health hazard quotient (HQ). The comparative LADD, ILCR and HQ for humans from Lindane through studied vegetables were presented in Table 1.

**Table 1**: Average daily intake of Lindane via vegetables and human health risk

| **Vegetables (n)** | **LADD ($mg\ kg^{-1}\ d^{-1}$)** | **ILCR** | **HQ** |
|---|---|---|---|
| Carrot (12) | $2.8x10^{-7}$-$4.1x10^{-7}$ ($3.4x10^{-7}$) | $3.1x10^{-7}$-$4.5x10^{-7}$ ($3.8x10^{-7}$) | $9.5x10^{-4}$-$1.4x10^{-3}$ ($1.1x10^{-3}$) |
| Cauliflower (13) | $8.4x10^{-8}$-$2.6x10^{-6}$ ($8.3x10^{-7}$) | $9.3x10^{-8}$-$2.8x10^{-6}$ ($9.2x10^{-7}$) | $2.8x10^{-4}$-$8.6x10^{-3}$ ($2.8x10^{-3}$) |
| Eggplant (9) | $2.9x10^{-8}$-$1.1x10^{-6}$ ($3.7x10^{-7}$) | $3.2x10^{-8}$-$1.2x10^{-6}$ ($4.1x10^{-7}$) | $9.8x10^{-5}$-$3.6x10^{-3}$ ($1.2x10^{-3}$) |
| Radish (7) | $9.8x10^{-8}$-$3.2x10^{-6}$ ($9.1x10^{-7}$) | $1.1x10^{-7}$-$3.5x10^{-6}$ ($1.0x10^{-6}$) | $3.3x10^{-4}$-$1.1x10^{-2}$ ($3.0x10^{-3}$) |
| Spinach (15) | $3.3x10^{-7}$-$5.5x10^{-7}$ ($4.8x10^{-7}$) | $3.7x10^{-7}$-$6.1x10^{-7}$ ($5.2x10^{-7}$) | $1.1x10^{-3}$-$1.8x10^{-3}$ ($1.6x10^{-3}$) |
| Tomato (8) | $4.3x10^{-8}$-$1.8x10^{-6}$ ($5.0x10^{-7}$) | $4.8x10^{-8}$-$1.9x10^{-6}$ ($5.5x10^{-7}$) | $1.4x10^{-4}$-$5.9x10^{-3}$ ($1.7x10^{-3}$) |
| All samples (64) | $2.9x10^{-8}$-$3.2x10^{-6}$ ($6.6x10^{-7}$) | $3.2x10^{-8}$-$3.5x10^{-6}$ ($7.3x10^{-7}$) | $9.8x10^{-5}$-$1.1x10^{-2}$ ($2.2x10^{-3}$) |

n=number of samples, average in parenthesis

The LADD of Lindane for all studied vegetables ranged between $2.9x10^{-8}$-$3.2x10^{-6}$ mg $kg^{-1}$ $d^{-1}$ with an average value of $6.6x10^{-7}$ mg $kg^{-1}$ $d^{-1}$. The average LADD of Lindane from carrot, cauliflower, eggplant, radish, spinach and tomato was $3.4x10^{-7}$ mg $kg^{-1}$ $d^{-1}$, $8.3x10^{-7}$ mg $kg^{-1}$ $d^{-1}$, $3.7x10^{-7}$ mg $kg^{-1}$ $d^{-1}$, $9.1x10^{-7}$ mg $kg^{-1}$ $d^{-1}$, $4.8x10^{-7}$ mg $kg^{-1}$ $d^{-1}$ and $5.0x10^{-7}$mg $kg^{-1}$ $d^{-1}$, respectively. These average daily intakes (ADIs) were within recommended acceptable limits for γ-HCH[10,13]. On the basis of estimated daily intake of Lindane, the estimated ILCR for all the samples was ranges between $3.2x10^{-8}$-$3.5x10^{-6}$ with an average of $7.3x10^{-7}$. This estimated ILCR was less the acceptable excess risk distribution range ($10^{-6}$-$10^{-4}$)[10].

Non-carcinogenic health effects (HQ) was evaluated by comparing the estimated average daily dose (mg $kg^{-1}$ $d^{-1}$) of the Lindane with the reference dose (RfD) (mg $kg^{-1}$ $d^{-1}$). The quantified HQ was ranged between $9.8x10^{-5}$-$1.1x10^{-2}$ with the mean of $2.2x10^{-3}$ for all the vegetables. However, average HQ through separate vegetables was $1.1x10^{-3}$, $2.8x10^{-3}$, $1.2x10^{-3}$, $3.0x10^{-3}$, $1.6x10^{-3}$ and $1.7x10^{-3}$, respectively for carrot, cauliflower, eggplant, radish, spinach and tomato. These estimated HI values were much lower than the acceptable safe risk level (HI≤1), indicating negligible risk.

**Conclusions**

Pesticide residues were found in all the monitored vegetable samples but never exceeded the recommended maximum residual limits (MRLs). Average daily dose were lower than reference doses and consequently, negligible health risk to humans may be concluded. The method of risk estimation involved a number of limitations which may lead to uncertainties in total risk. For example, in this study of risk analysis did not consider different ages, because childhood may have a greater probability of producing risk than exposure in adulthood. In addition, human body weight and possible synergistic effects of other toxic chemicals could lead to uncertainties. Therefore, a more comprehensive study in detail is required, including other persistent and toxic pollutants such as PCBs, dioxins and heavy metals.

**Acknowledgements**
Authors are highly thankful to the Chairman and Member Secretary, Central Pollution Control Board for support and encouragement to conduct this study.

**References**
1. ATSDR. 2005. Toxicological profile for hexachlorocyclohexanes.
2. UNEP. 2009. UNEP/POPS/COP.4/38, 8 May 2009; Accessed on 20.2012 at: http://www.POPs.int/.
3. Van den Berg H. 2009. Environ Health Persp 117(11):1656-1663.
4. Jit S, Dadhwal M, Kumari H, *et al*. 2011. Environ Sci Pollut Res., 18:586–597.
5. Srivastava AK, Trivedi P, Srivastava MK, *et al*. 2011. Environ Monit Assess, 176:465–472.
6. Bankar R, Ray AK, Kumar A, Adeppa K, Puri S. 2012. Acta Biologica Indica, 1(1):77-80.
7. Gowda SRA and Somashekar RK. 2012. Bull Environ Contam Toxicol., 89:626–632.
8. Kumari B & Kathpal TS. 2009. Environ Monit Assess, 151:19–26.
9. USEPA. 1989. Risk Assessment Guidance for Superfund. Human Health Evaluation Manual (Part A). EPA 540-1-89-002, United States Environmental Protection Agency, Washington, DC.
10. USEPA. Accessed on 20.10.2012, http://wwwepagov/reg3hwmd/risk/human.
11. EC Directive. 2008. Commission Regulation (EC) No 149/2008. Amending Regulation (EC) No 396/. Available on-line at: http://www.efsa.europa.eu or http://ec.europa.eu/sanco pesticide/publish/index.cfm.
12. FSSAI (Food Safety Standards Authority of India). Food Safety and Standards regulations, 2013, http://www.fssai.gov.in.
13. WHO (World Health Organization). 2009. Joint Meeting on Pesticide Residues (JMPR) through 2009: http://www.who.int.