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Status of HCH Residues in Indian Medicinal Plant Materials

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Herbal materials are being used for preparation of ayurvedic medicines and tonics since ancient periods and ayurveda workers left no stone unturned to explore the properties, pharmacology and toxicology of herbal materials (Ensminger et al 1994). At present herbal ayurvedic medicines are in great demand around the globe as an alternate system because of their low toxicity and diversified uses against various diseases (Warrier et al 1996). India exports a significant quantity of ayurvedic medicines in different parts of the world. Further, the enormous use of organochlorine pesticides in developing countries has been of serious concern because of their persistent nature. The presence of Organochlorine Pesticides (OCPs) in terrestrial and aquatic environment may lead to toxicological implications (Aruda et al. 1988; Cochieri and Arnese 1988; Sarkar and Gupta 1988; Dikshith et al. 1989). Pesticides in large amounts, are used in agriculture and public health programmes every year (Ciers 1998). Continuous use of OCPs have led to their presence in water, soil, air, crops and biological tissues. This could be possible source of contamination of the herbs used in herbal ayurvedic preparations, used as tonics, drugs, toiletries, cosmetics etc. Pesticide residue analysis has been done on several food/food products (Raizada et al. 1998; Bhattacharyya 2003; Kalra 2003; Mukherjee 2003; Raghupathy 2003). Organochlorine pesticide residues have been reported in herbal products from Poland and Germany. (Pluta 1989; Beneck et al 1989). The authors have reported the residue level of OCPs in some of the ayurvedic preparations and Indian spices being used as medicines and taste and flavoring materials in food stuffs(Srivastava et al2000 and 2001). There is inadequate information on the presence of OCPs in various varieties of herbal materials used for preparation of ayurvedic medicines. The present study deals with the analysis of OCP (HCH and DDT) residues in above medicinal plant materials to evaluate the present level of these commonly used persistent pesticides.

MATERIALS AND METHODS

All the solvents used in extraction and cleanup processes were of HPLC grade procured from M/s Spectrochem Pvt. Ltd. Bombay. Activated charcoal, sodium sulfate, sodium chloride and other chemicals, used in the study were procured from E. Merk India Ltd., BDH, and Glindia Ltd. Five samples of each herbal

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Table 1 List of the Plant materials studied with their habitat and uses.

Name of plant Hindi/Botanical	**Habitat**	**Useful and studied Part**	**Uses**
Amla *(Emblic myrobala,)*	Deciduous forests, hill slopes also in plains	Fruits,	Fruits useful in diabetes cough asthma dyspepsia, flatulence, hyperacidity, peptic ulcers, leprosy
Arjun *(Terminalia arjuna)*	Throughout India	Bark	Cardiac problems, ulcers, diabetes, anemia, asthma, bronchitis
Ashwagandha *(Withania somnifera)*	Through out drier part of India	Roots	Tissue building and nervous breakdown,
Bahera *(Terminalia bellirica)*	Deciduous forest	Fruit	Cough, bronchitis, vomiting, skin diseases, leprosy fever, and general debility
Brahmi *(Centella asiatica)*	Through out India	Whole plant	Insomnia, cardiac debility, abdominal disorder due to dysentery, fever
Butch *(Acorus calamus linn)*	Through out India in areas elevated up to 1800m	Rhizomes	Mental disorders, gout, skin diseases, helminthiasis, cough, bronchitis
Elaichi *(Elettaria cardamomum maton)*	South India	Seeds	Seeds useful in digestion cardio tonic. oil useful in asthma, bronchitis and other pharmaceutical preparations
Guggul *(Commiphora mukul)*	Arind rocky tracks of Rajputan, Mysore, Simla, Baluchistan	Resinous gum	Expectorant, antiseptic, nerve tonic, ,antispasmodic, cough, asthma, bronchitis, hepatic disorder
Gulab *(Rosa centifolia)*	Cultivated through out India	Flowers,	Flowers are atrin- gebt, sweet, cooling, and aromatic cardio tonic, expectorant, digestive and tonic.

Table 1 continued

Harr *(Terminalia chebula-retz)*	Deciduous forests on dry slopes of West Bengal & and Tamilnadu	Fruits	Useful in wounds, jaundice, cough and general debility and as laxative
Indrayan (not known)	Through out India in warm areas	Roots	Useful in tumors, leucoderma, asthma, bronchitis, jaundice, constipation
Lavang *(Syzygium aromaticum*	South India	Dried flower buds	Useful in cough, asthma, burning sensation fever, dental cavities and tuberculosis
Maquoy *(Solanum nigrum linn)*	Weed in dry part of India	Whole plant	Leaves used for rheumatic gouty joints, and skin diseases . Berries and flowers used in cough, bronchitis, pulmonary tuberculosis, fever , gastrihelicosis
Mulethi *(Glycyrrhiz a glabralin)*	Cultivated in Punjab and sub-Himalayan tracts	Roots	Hyper dyponia, cough, gastrological, bronchitis cephalgia, fever, gastric ulcers
Munakka *(Vitis vinifera)*	Central parts of India	Fruits	Old age sickness, and general tonic
Neem *(Azadiracht a indica)*	Through out India in deciduous forests, also idly cultivated	Bark	Skin diseases, eczema, leucoderma, malaria, wounds, ulcers, tumor, intestinal worms, tuberculosis
Punarnava *(Boerhaavia diffusa linn)*	Found as weed in waste lands	Whole plants	Laxative ,useful in jaundice liver inflammations anemia and general debility
Rasana *(Alpinia galanga (linn)*	Through out western coast of India	Rhizo mes	Rhizomes useful in cough, asthma, bronchitis, rheumatoid arthritis, intermittent fever
Satavar *(Asparagus recemosus)*	Through out India up to 1400m	Tubers	Nerves disorders, inflammations, hypertension, hepatopathy, epilepsy
Vidhara *(Pueraria tuberose)*	Through-out India	Tuber ous roots	Arthritis, burning sensation, constipation, cardiac debility, leprosy, tuberculosis, cough and general debility

materials were collected from local markets of Lucknow(Table 1). Analysis was carried out as per AOAC (1995). Each sample (50g) in triplicate was soaked with 100ml (n-hexane : acetonitrile mixture 1:3), kept over night and shaken for 30 min. After decanting, organic phase was separated from polar phase. Polar phase was further extracted 3 times with 50ml n-hexane in separatory funnel. Total pool of hexane extract was washed with distilled water 3 times. Washed hexane extract was passed through charcoal and anhydrous sodium sulfate column. The clean hexane extract was concentrated, made 5 ml and analysed on precalibrated GC machine (Nucon 5765) equiped with ^{63} Ni electron capture detector. A glass column (1.5m x 2mm id) packed with 1.5%OV-17 + 1.95% Qf-1 on 100-120 mesh chromosorb WHP was used. Operation temperatures were programmed at 195, 200, 220^{o}C for column, injector and detector respectively. Purified nitrogen gas passing through silica gel and molecular sieves was used as carrier gas at flow rate of 60ml/min. Periodically procedural blanks were used to check cross contamination. Confirmation was done by dual columns in some random selected samples. Recovery studies with purified samples indicated that overall recovery value exceeded 80%. Identification and quantification were accomplished using known amount of external standard received from US EPA, Pesticides and Industrial Chemicals repository (MD-8) Research triangle, NC, USA.

RESULTS AND DISCUSSION

Results revealed that level of HCH was higher than DDT in almost all the items except *Arjun, Baher, Buch, Harr, Rasna and Satavar*. Highest level of HCH was found in *Elaichi* ie 25.46 µg/kg (Table 2). Lowest level of HCH was recorded in *Arjun* which was 0.63 µg/kg. alpha HCH (7.66 µg/kg) and gamma HCH (15.66 µg/kg) where higher in *Elaichi*. However, higher level of beta HCH and delta HCH were found in *Ashwagandha* and *Bahera* respectively.

DDT was found to be maximum in *Bahera* (14.20 µg/kg) and minimum in *Arjun* (0.22 µg/kg) (Table 3). Isomers of DDT like opDDT and ppDDT showed almost a comparable presence in both *Neem* and *Bahera* which contained maximum 6.99 and 9.90 µg/kg respectively. However, the metabolite ppDDE, was maximum 2.68 µg/kg in *Rasna*. The presence of DDT residues significantly higher than DDE residues in some samples suggest recent contamination or usage in those samples. *Guggul* contained maximum level of ppDDD (8.99 µg/kg). Though only 2 samples contained pp,DDD. It is also noticeable that in *Bahera, Harr, Rasna and Satawar* the level of DDT was higher than level of HCH.

The relationship among the presence of maximum level of OCPs in various harbal materials was HCH > DDT There is no MRL/Tolerance limit of organochlorine pesticides in herbal materials prescribed in PFA (1954) or Codex (1996). Sullivan (1980) reported the total level of DDT and BHC below 500 µg/kg in spices imported into United States, and concluded that the levels of above pesticides in general are sufficiently low and there is no cause of alarm.

Our studies have also shown low level i.e.25.46 µg/kg (BHC) and 14.20 µg/kg (DDT) in Indian herbal materials. It is well known that organochlorine pesticides are lipophillic in nature and accumulate in fat. It could be one of the reasons for

Table 2 Level of HCH isomers (µg/kg)in herbal materials.

Name of plant	α-HCH	β-HCH	γ-HCH	δ-HCH	Total HCH
Amla	0.40 (0.09-0.71)	ND	1.60 (0.83-1.81)	0.96 (0.12-1.81)	2.52 (1.00-4.01)
Arjun	0.15 (ND-0.31)	ND	0.48 (ND-0.86)	ND	0.63 (ND-1.18)
Ashwa-gandha	0.69 (0.55-0.84)	4.63 (1.25-8.00)	1.64 (1.45-1.84)	ND	5.92 (1.84 -10.00)
Bahera	0.91 (ND-1.76)	ND	0.51 (ND- 1.04)	1.49 (ND-2.58)	2.50 (ND-5.39)
Bramhi	0.62 (0.61-0.62)	0.60 (0.41-0.79)	2.28 (2.24-2.32)	ND	3.40 (3.34-3.65)
Butch	0.94 (0.27- 1.62)	0.57 (0.23-0.90)	1.30 (1.08-1.52)	ND	2.87 (1.71-4.0)
Elaichi	7.66 (ND-9.61)	ND	15.66 (ND-18.92)	1.23 (ND-1.38)	25.46 (ND-29.91)
Guggul	7.2 (4.88-9.65)	ND	3.85 (3.77- 3.92)	0.60 (0.57-0.64)	11.72 (9.38-14.05)
Gulab	1.76 (0.65-2.87	ND	3.04 (1.35-4.74	1.08 (0.38-1.78)	5.89 (3.78-7.10)
Harr	0.75 (0.41-1.09)	ND	1.47 (0.83 -2.10)	0.28 (0.14-0.42)	2.50 (1.38-3.62)
Indrayan	1.98 (0.81-3.16	ND	5.12 (4.68-5.55)	0.37 (ND-0.37)	7.75 (6.42-9.08)
Lavang	9.34 (ND-11.68)	ND	8.46 (ND-11.09)	0.98 (ND-1.41)	20.35 (ND-24.19)
Maquaya	0.48 (0.42-0.54)	0.73 (0.62-0.84)	1.51 (1.37-1.66	ND	2.73 (2.41-3.05)
Mulethi	0.39 (0.30 - 0.48)	0.01 (ND-0.01)	0.90 (0.78-1.02)	1.26 (1.23-1.28)	2.55 (2.37-2.72)
Munnakka	1.74 (ND-3.05)	ND	1.45 (ND-2.30)	0.20 (ND-0.33)	3.92 (ND-5.88)
Neem	3.37 (3.05-3.68)	ND	2.07 (1.10-2.16)	ND	5.06 (5.21-6.90)
Punernaw a	0.4137 (0.48-0.35)	0.83 (0.66-1.00)	1.41 (1.21-1.60)	ND	2.65 (2.22-3.09)
Rasna	1.82 (0.74-2.91)	0.93 (ND-0.93)	2.02 (1.66-2.39)	0.40 (0.27-0.53)	4.72 (4.59-4.85)
Satavar	0.24 (ND-0.52)	ND	0.41 (ND-0.78)	1.08 (ND-2.18)	1.76 (ND-3.49)
Vidhara	0.59 (0.53-0.66)	0.51 (0.30-0.73)	1.78 (1.45-2.11)	ND	2.88 (2.27-3.49)

ND = Not detected or < 0.01; Values are the mean of 5 samples; Values in parenthesis are range

Table 3 Level of DDT and its metabolites (μg /kg) in herbal materials.

Name of plant	ppDDE	opDDT	ppDDD	ppDDT	Total DDT
Amla	0.53 (0.38-0.68)	ND	ND	ND	0.53 (0.38-0.69)
Arjun	0.07 (0.06-0.09)	0.17 (ND-0.33)	ND	ND	0.22 (0.09-0.39)
Ashwa-gandha	1.54 (1.05-2.12)	1.2127 (0.82-1.61)	ND	0.74 (0.64-0.84)	3.49 (2.51-4.46)
Behera	0.13 (ND-0.26)	3.50 (ND-6.87)	ND	9.90 (ND-18.97)	14.20 (ND-26.10)
Brahmi	0.08 (ND-0.08)	0.81 (0.65-0.98)	ND	0.32 (0.26-0.70)	1.54 (1.39-1.67)
Butch	0.057 (ND-0.09)	3.23 (ND-5.24)	ND	1.67 (ND-2.73)	4.03 (0.09-7.97)
Elaichi	0.06 ND-0.09)	4.88 (ND-8.60)	ND	2.90 (ND-4.54)	8.36 (ND-13.23)
Guggul	0.13 (ND-0.21)	8.91 (ND-18.08	8.91 (ND-14.3)	2.98 (ND-4.49)	10.67 (ND-18.73)
Gulab	0.25 (0.09-0.40)	1.55 (ND-3.12)	ND	2.93 (ND-5.53)	4.67 (ND-9.06)
Harr	1.26 (0.40-2.12)	4.22 (ND-8.25)	ND	0.76 (ND-1.39)	6.08 (0.40-11.76)
Indrayan	0.23 (ND-0.38)	1.99 (ND-2.66)	ND	1.77 (ND-2.67)	3.16 (ND-5.72)
Lavang	ND	3.68 (ND-8.51)	ND	2.96 (ND-4.86)	8.36 (ND-13.38)
Maquya	0.86 (0.74 -0.97)	0.87 (0.73-1.02)	ND	0.57 (0.24 -0.89)	2.30 (1.71-2.89)
Mulati	0.43 (0.35-0.50	ND	ND	ND	0.43 (0.35 -0.50
Munakka	ND	1.0145 (ND-2.72)	ND	ND	1.01 (ND-2.72)
Neem	ND	6.99 (ND-0.32)	1.80 (ND-2.57)	0.44 (ND-0.62)	3.27 (2.57-10.94)
Punernawa	0.73 (0.63-0.83)	0.44 (ND-0.74)	ND	0.44 (ND-0.74)	1.47 (0.63-2.31)
Rasna	2.68 (ND-4.13)	4.81 (ND-9.04)	ND	2.89 ND-4.85)	9.56 (ND-4.13)
Satavar	0.11 (0.94-1.33)	0.38 (ND-0.94)	ND	0.37 (ND-0.72-	0.58 (0.09-1.074)
Vidhara	0.12 (0.08-0.16)	0.83 (0.72-0.93)	ND	0.0.35 (ND-0.75)	1.32 (0.80-1.84)

ND= Not detected or < 0.01; Values are the mean of 5 samples; Values in parenthesis are range

getting the maximum level of BHC and DDT in *Elaichi and Bahera* fruits which contain oil in the seeds. OCPs may find their way into human system through food and water. The organochlorine pesticide residues have also been detected in human diet (Kannan et al. 1992) , drinking water (Dikshith et al. 1990) and in

herbal preparations and spices (Srivastava et al 2000, 2001) . Pesticides are known to enter in plant products from contamination via spillage and volatalisation of residues from pesticide treated soils or storage. Though, the pesticides like HCH and DDT are well known toxic chemicals (WHO 1974; Bulger and Kupfer 1985; Hayes 1991), their residues were found at very low level in all the samples of herbal materials analysed. This level may even go much lower after processing for ayurvedic preparations as drugs or tonics . It is known that certain physical and chemical conditions like washing, heat treatment, steaming and treatment with supercritical carbon dioxide are known to reduce the level of pesticides (Stahl and Rau 1984; Nash 1984; Srivastava and Nath 1990). Even then, the herbal materials should be monitored periodically as these are exported to various countries.

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