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Pesticide Residues in Honey Samples from Himachal Pradesh (India)

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Abstract Honey, being a natural product manufactured by honey bees is considered to be free from any extraneous material. The over-reliance on pesticides caused several environmental problems including pesticide residues in food. This constitutes a potential risk for human health, because of their sub acute and chronic toxicity. Therefore this study was carried out to know the extent of pesticide residue in honey produced in the various parts of Himachal Pradesh. Among different pesticides analysed in honey; HCH and its isomers were the most frequently detected followed by DDT and its isomers. Of the studied synthetic pyrethroids, only cypermethrin was found in honey samples. Residues of organophosphates viz. acephate, chlorpyriphos, ethion and monocrotophos were not detected, however malathion's residue was found exceeding the MRL (5 ppb) proposed by the Ministry of Commerce, Government of India. More over honey from natural vegetation contained lesser residues. It can be concluded that honey from Himachal Pradesh had low pesticide residues.

Keywords Honey · Pesticide residues · GC · Multiresidue

Pesticides play a vital role and their use is inimical in agriculture. In India, the most widely used pesticides are organophosphorus, synthetic pyrethroids and carbamates, which have almost completely replaced organochlorine pesticides (Porrini et al. 2002). The extensive distribution of these groups of pesticides caused several problems to apiculture industry including residues in hive products (honey, wax and propolis etc.). These residues enter in to the honey and finally to the consumer (Fong et al. 1999; Porrini et al. 2002). This constitutes a potential risk for human health, because of their sub acute and chronic toxicity. Honey, being a natural product is considered to be free from any extraneous material but residues of pesticides were recorded by several workers (Campas and Rempel 1976; Estep et al. 1977; Gayger and Dustmann 1985; Al-Rifai and Akkel 1997; Sarfraz Khan et al. 2004).

MRL's (Maximum Residue Limit) for acaricides have been framed by some countries but for insecticides these are neither included neither in the *Codex Alimentarius* nor in the EU (European Union) (Al-Rifai and Akkel 1997; Blasco et al. 2003; Sarfraz Khan et al. 2004). Different national regulations have established maximum concentrations of pesticide residues permitted in honey, but the lack of homogeneity causes problems in the international marketing and trade.

Pesticides consumption in the state of Himachal Pradesh is very low compared to other states of India. So it is speculated that honey collected from the state is free from the pesticide residues. In contrary to this Sharma and Kashyap (2002) recorded pesticide residues in honey. So the present study was planned (i) to analyse honey samples collected from the state more critically with a view to eliminate the chance of rejection of any honey consignment in the international market on the basis of pesticide residues and (ii) to provide the monitoring data to avoid any health hazard to consumer.

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Materials and Methods

Standard pesticide solutions were obtained from Toxicology Laboratory, Punjab Agricultural University, Ludhiana, Punjab (India). Solvents like acetone (A.R.), methanol (A.R.), *n*-hexane (A.R.) were obtained from Merck (India) and distilled in vacuo in glass distillation. Anhydrous sodium sulphate, florisil, silica gel were purchased from Ranbaxy (India). Stock solutions (1,000 mg L^{-1}) were prepared in acetone and stored at −4°C in deep freezer.

Fifty-one honey samples (500 g) were collected from commercial beekeepers of the state along with information on the various aspects regarding origin and honeybee species. An analytic method based on liquid–liquid extraction (LLE) was followed for the residue estimation of organochlorine, cyclodiene, synthetic pyrethroid and organophosphorus pesticides due to the advantage of simplicity and ability to handle large sample volumes.

Different methods (Ogota and Bevenue 1973; Chawla and Goyal 1988; Karak et al. 1999) were reviewed for simultaneous estimation of organochlorine, cyclodiene and synthetic pyrethroid pesticide's residues and used after slight modification. A sample of honey (50 g) was taken in 250 mL conical flask and mixed with 100 mL each of distilled water and methanol. This mixture was shaken for 3 h on a mechanical shaker and filtered through Whatman No. 1 filter paper. For clean up, the above extract was transferred to a separating funnel of 500 mL capacity and 100 mL of *n*-hexane was added to it. After shaking the funnel for 15 s, it was kept for separation of layers. The lower layer was drained out in to a conical flask and the organic layer was collected over anhydrous sodium sulphate. The process was repeated twice. This solution was concentrated to 1–2 mL through vacuum rotary evaporator (Buchhi type) and transferred to glass column pre packed with activated florisil:silical gel mixture (1:1). Distilled *n*-hexane was used as eluant and final volume was made up to 30 mL for each sample.

For the analysis of organophosphates; methods of Sharma and Kashyap 2002; Sarfraz Khan et al. 2004 were modified and used. A representative 10 g honey sample was diluted with 100 mL of 4% aqueous solution of sodium sulphate in a 250 mL conical flask. This mixture was shaken for 1 h on a mechanical shaker and filtered through Whatman No. 1 filter paper. Each sample of extracted honey with distilled water (50 mL) was transferred to a separating funnel (250 mL) and extracted four times with *n*-hexane (50 mL). Each time the hexane phase was transferred to a tube and centrifuged for 5 min at 5,000 rpm in order to break up the emulsion that appeared in water and hexane inter-phase. Afterwards, the extract was dried over anhydrous sodium sulphate (15 g). This solution was concentrated to 1–2 mL through vacuum rotary evaporator at 35°C. Concentrate was transferred to glass column pre-packed with activated florisil:silical gel mixture (1:1). Distilled *n*-hexane was used as eluant. The eluant was collected in flask and evaporated to dryness in a vacuum rotary pump (at 35°C). The dried residues were diluted with hexane to 5 mL and stored in clean reagent bottles.

The detection of pesticide residues was accomplished by gas chromatography (GC). Auto GC, PERKIN ELMER, USA make, equipped with ECD, flexible capillary columns BP225 (composed of 50% cyanopropyl + 50% diethyl silixane, 50 mt in length with 0.5 μ film thickness, 0.53 mm i.d. and 0.78 mm o.d.) and autosampler was used. It was connected with terminal integrator model PE 1022 and printer model OKIDATA-320. The column temperature was programmed as 160°C (0.00 min) → 3°C min^{-1} → 240°C (32.00 min) with oven maxima of 450°C, detector temperature of 300°C. Sample was injected in split mode. Nitrogen (N_2) was used as carrier gas at the flow rate of 30.0 mL min^{-1} for organochlorine, cyclodiene and synthetic pyrethroid pesticide's residue estimation.

Whereas, for organophosphate pesticides NPD was used with BPX5 column (containing 5% phenyl equivalent modified silicone, 25 m long having 3.0 μ film thickness, 0.32 mm i.d. and 0.43 mm o.d.). Temperature of the column was programmed as 160°C (0.00 min) → 3°C min^{-1} → 220°C (10.00 min) → 3°C min^{-1} → 240°C (10.00 min). Oven maxima and detector temperatures were 450 and 300°C, respectively. Split valve was on. Carrier gas (N_2), hydrogen (H_2) gas and zero gas (O_2) were used at the flow rates of 30.0, 5.0 and 80.0 mL min^{-1}.

Recovery experiments were also conducted, in triplicate, at various fortification levels, by adding known volumes of pesticides. The blank samples were also processed in the same way to find out the differences, if any. Recoveries for organochlorine and cyclodiene pesticides varied between 83.66% ± 0.15% for dicofol to 91.33% ± 0.10% for β-endosulfan. Percent recoveries for the synthetic pyrethroid pesticides varied between 87.83 ± 0.11 and 90.33 ± 0.23 for deltamethrin and fenvalerate, respectively. Organophosphorus pesticides were recovered in the range between 76.33 ± 0.15 and 83.66% ± 0.03% for malathion and dimethoate, respectively.

The detection limits were found to be 0.05 ppb for HCH isomers, 0.10 ppb for *pp′*, *op′* DDT, endosulfan I and II, 0.15 ppb for *pp′* DDT and 0.50 ppb for dicofol. For synthetic pyrethroid these values were recorded to be 0.50 ppb for lambda cyhalothrin I and II and fenvalerate I and II. Values for detection limits were comparatively higher for cypermethrin I and II (1.0 ppb) and deltamethrin (2.0 ppb). Different organophosphorus pesticides have detection limits to the tune of 2.0 for dimethoate and ethion whereas, it was 5.0 ppb for acephate, monocrotophos, malathion, chlorpyriphos and quinalphos.

Table 1 Level of organochlorine pesticide residues (μg kg^{-1}) in different samples of honey

Sample no.	Sample character	*p,p′* DDE	*p,p′* DDD/ TDE	*o,p′* DDT	*p,p′* DDT	α-HCH	*β*-HCH	*γ*-HCH
1.	$F_8D_4S_3$	BDL	BDL	BDL	BDL	1.401 ± 0.309	BDL	2.970 ± 0.058
2.	$F_3D_7S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
3.	$F_8D_7S_3$	BDL	BDL	BDL	BDL	BDL	1.650 ± 0.047	0.760 ± 0.085
4.	$F_2D_7S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
5.	$F_6D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
6.	$F_6D_1S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
7.	$F_6D_4S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
8.	$F_7D_6S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
9.	$F_7D_9S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10.	$F_7D_9S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
11.	$F_8D_5S_1$	0.522 ± 0.032	0.985 ± 0.045	BDL	BDL	0.095 ± 0.005	BDL	0.463 ± 0.057
12.	$F_6D_5S_2$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
13.	$F_6D_5S_1$	BDL	0.380 ± 0.040	BDL	2.088 ± 1.044	BDL	BDL	BDL
14.	$F_6D_5S_1$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
15.	$F_1D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
16.	$F_7D_6S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
17.	$F_6D_8S_3$	BDL	BDL	BDL	BDL	BDL	BDL	0.369 ± 0.056
18.	$F_6D_8S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
19.	$F_6D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
20.	$F_6D_5S_3$	BDL	BDL	BDL	BDL	0.262 ± 0.021	BDL	0.126 ± 0.017
21.	$F_4D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
22.	$F_6D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
23.	$F_4D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
24.	$F_8D_5S_3$	BDL	BDL	BDL	BDL	0.602 ± 0.059	BDL	1.814 ± 0.259
25.	$F_3D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
26.	$F_8D_5S_3$	0.290 ± 0.034	BDL	BDL	0.271 ± 0.025	BDL	BDL	BDL
27.	$F_5D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
28.	$F_8D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
29.	$F_8D_5S_3$	BDL	BDL	BDL	BDL	BDL	0.139 ± 0.008	0.804 ± 0.121
30.	$F_1D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
31.	$F_8D_5S_3$	BDL	0.902 ± 0.099	0.415 ± 0.060	BDL	BDL	BDL	BDL
32.	$F_8D_5S_1$	BDL	BDL	BDL	BDL	0.113 ± 0.002	BDL	0.140 ± 0.015
33.	$F_6D_5S_3$	BDL	BDL	0.377 ± 0.030	3.052 ± 0.014	BDL	BDL	BDL
34.	$F_6D_5S_2$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
35.	$F_6D_5S_1$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
36.	$F_6D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
37.	$F_6D_5S_1$	BDL	BDL	BDL	BDL	0.704 ± 0.012	0.232 ± 0.083	1.513 ± 0.134
38.	$F_9D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
39.	$F_9D_5S_3$	BDL	3.701 ± 0.403	BDL	BDL	BDL	BDL	BDL
40.	$F_3D_7S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
41.	$F_8D_7S_3$	0.639 ± 0.007	BDL	1.891 ± 0.171	BDL	BDL	BDL	BDL
42.	$F_2D_7S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
43.	$F_9D_{11}S_4$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
44.	$F_8D_7S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
45.	$F_9D_1S_4$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
46.	$F_9D_2S_1$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
47.	$F_9D_2S_1$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
48.	$F_9D_{10}S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Table 1 continued

Sample no.	Sample character	*p,p′* DDE	*p,p′* DDD/ TDE	*o,p′* DDT	*p,p′* DDT	α-HCH	*β*-HCH	*γ*-HCH
49.	$F_9D_{10}S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
50.	$F_9D_3S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
51.	$F_6D_5S_3$	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Range		BDL-0.639	BDL-3.701	BDL-1.891	BDL-3.052	BDL-1.401	BDL-1.650	BDL-2.970

BDL, below detectable limit. Values following ± are SE of mean

F (Floral source) F_1 Acacia; F_2 Berseem; F_3 Eucalyptus; F_4 Honey Dew; F_5 Litchi; F_6 Multiflora; F_7 Plectranthus; F_8 Rapeseed; F_9 Unknown

D (Districts) D_1 Chamba; D_2 Bilaspur; D_3 Hamirpur; D_4 Kullu; D_5 Kangra; D_6 Kinnaur; D_7 Sirmour; D_8 Solan; D_9 Shimla; D_{10} Una; D_{11} Unknown (market etc.)

S (Honey bee species) S_1 *Apis cerana*; S_2 *Apis dorsata*; S_3 *Apis mellifera*; S_4 unknown

Table 2 Level of cyclodienes and synthetic pyrethroids pesticide residues (ppb) in different samples of honey

Sample no[a]	α-Endosulfan	*β*-Endosulfan	Dicofol	Cypermethrin	Deltamethrin	Fenvalerate	Lambda cyhalothrin
1.	BDL	BDL	BDL	10.234 ± 0.010	BDL	BDL	BDL
11.	BDL	BDL	BDL	5.550 ± 0.887	BDL	BDL	BDL
20.	2.731 ± 0.396	2.038 ± 0.313	BDL	BDL	BDL	BDL	BDL
27.	BDL	BDL	2.226 ± 0.024	BDL	BDL	BDL	BDL
28.	0.645 ± 0.057	BDL	BDL	BDL	BDL	BDL	BDL
29.	BDL	BDL	BDL	2.856 ± 0.685	BDL	BDL	BDL
31.	2.122 ± 0.107	BDL	BDL	BDL	BDL	BDL	BDL
38.	BDL	BDL	BDL	BDL	6.663 ± 1.020	0.696 ± 0.137	BDL
41.	BDL	BDL	BDL	BDL	BDL	BDL	0.765 ± 0.004
45.	0.258 ± 0.002	BDL	BDL	BDL	BDL	BDL	BDL
50.	BDL	BDL	BDL	BDL	BDL	BDL	0.636 ± 0.034
51.	1.990 ± 0.031	2.502 ± 0.141	BDL	BDL	BDL	BDL	BDL
Range	BDL-2.731	BDL-2.502	BDL-2.226	BDL-10.234	BDL-663	BDL-0.696	BDL-0.765

ppb, Parts per billion; BDL, below detectable limit. Values following ± are SE of mean

[a] Samples no's 2–10, 12–19, 21–26, 30, 32–37, 39–40, 42–44, 46–49 had residues BDL

Table 3 Level of organophosphorus pesticide residues (ppb)[a] in different samples of honey

Sample no[b]	Dimethoate	Malathion	Quinalphos
11.	BDL	11.077 ± 1.157	BDL
23.	BDL	9.187 ± 1.412	BDL
24.	BDL	BDL	10.270 ± 0.319
26.	BDL	6.663 ± 1.020	BDL
28.	8.667 ± 0.882	BDL	BDL
31.	BDL	BDL	8.907 ± 0.689
35.	2.448 ± 0.221	BDL	BDL
44.	3.265 ± 0.002	BDL	BDL
Range	BDL-8.667	BDL-11.077	BDL-10.270

ppb, parts per billion; BDL, below detection limit. Values following ± are SE of mean

[a] Residues of acephate, chlorpyrihos, ethion and monocrotophos were BDL

[b] Sample no's 1–10, 12–22, 25, 27, 29–30, 32–34, 36–43 and 45–51 had residues BDL

Results and Discussion

All 51 honey samples were analysed for the simultaneous detection of organochlorine, cyclodiene and synthetic pyrethroids pesticides residues on GC (Tables 1, 2). It was found that 18 honey samples (35.29%) were contaminated with the residues of organochlorine and cyclodiene pesticides. HCH and its various isomers were found to be the major contaminant (17.64%) of honey followed by DDT and its isomers (13.72%). Although the use of persistent organic pollutants (POP's) viz. DDT and HCH have been banned in India for use in agriculture and put under restricted use for decades, even then their residues were still detected. Their regular contamination in honey may be attributed to their stable nature and very long persistence in soil and water which was source of honey contamination (Etto 1974). Besides these two organochlorine pesticides, dicofol was found only in litchi (*Litchi sinensis*) honey where it was used against mite pests.

Residues of endosulfan and its isomers were present in 7.84% honey samples. Comparisons were also made with the difference in honey bee and type of floral origin. It was recorded that commercial apiary honeys (collected by *A. mellifera*) were more contaminated with pesticide residues at the higher concentrations (31.37%) than were honey produced by *A. cerana* (9.80%). It was also found that a majority of contaminated samples were of mustard and rapeseed as floral origin (90.90%) followed by multifloral honeys (41.17%). Results might be due to the differential forage resources and foraging ranges of *A. mellifera* compared to other honey bee species viz. *A. cerana* and *A. dorsata* (Sarfraz Khan et al. 2004). *A. cerana* and *A. dorsata* were reported to forage on wide range of plants including field crops but rely more on wild flora where insecticide generally were never applied so the percent honey contamination was less. Conversely, the domesticated bees; *A. mellifera* usually forage on cultivated crops where the use of pesticides was much more common which resulted in higher contamination of honey samples (Jhansi et al. 1991). Hence, depending upon the floral resources, low or no contamination is found in honeys from *A. cerana* and *A. dorsata*.

Synthetic pyrethroids are the pesticides largely used to manage lepidopteran pests of crops. Among four pyrethroid pesticides analysed (Table 2) residues of cypermethrin were found in three, lambda cyhalothrin in two and of deltamethrin and fenvalerate each in one honey samples. It was also observed that mustard and market honey samples contained the residues of above said pesticides. The contamination might be due to the usual practice of spraying synthetic pyrethroids on these crops to manage insect pests.

Organophosphorus pesticides residues were also least observed in honey samples (Table 3). The residues of acephate, chlorpyriphos, ethion and monocrotophos were not detected in any of the honey samples whereas, residues of dimethoate were found in three honey samples (two mustard honey and one multifloral honey), malathion also in three honey samples (two mustard honey and one honey dew) and quinalphos in two honey samples (both mustard honey). Contamination of mustard honey was higher which might be due to the contamination of nectar and pollen either by direct application or drift of pesticides to flowering plants (Woodwell et al. 1971).

Form the Table 4 it was found that 49.02% were contaminated with the residues of either one or other pesticide. But if we compare the amount of residues with the MRL's as proposed by the Ministry of Commerce, Government of India for the Honey Export (Anonymous 2002), it was

Table 4 Percent honey samples contamination due to pesticides

Pesticides residues detected	Mean	Range	MRL[a] (μg kg^{-1})	Frequency	Percent contamination	Percent above MRL
p,p′ DDE	0.484 ± 0.102	BDL-0.639	50.0	3	5.88	0.00
p,p′ DDD/TDE	1.492 ± 0.864	BDL-3.701	50.0	4	7.84	0.00
o,p′ DDT	0.894 ± 0.498	BDL-1.891	50.0	3	5.88	0.00
p,p′ DDT	1.804 ± 0.815	BDL-3.052	50.0	3	5.88	0.00
α-HCH	0.530 ± 0.286	BDL-1.401	5.0	6	11.76	0.00
β-HCH	0.674 ± 0.489	BDL-1.650	5.0	3	5.88	0.00
γ-HCH	0.985 ± 0.543	BDL-2.970	5.0	9	17.65	0.00
α-Endosulfan	1.549 ± 0.605	BDL-2.731	100.0	5	9.80	0.00
β-Endosulfan	2.270 ± 0.189	BDL-2.502	–	2	3.92	–
Dicofol	2.226 ± 0.024	BDL-2.226	50.0	1	1.96	0.00
Cypermethrin	6.213 ± 2.155	BDL-10.234	50.0	3	5.88	0.00
Deltamethrin	6.663 ± 1.020	BDL-6.663	50.0	1	1.96	0.00
Fenvalerate	0.696 ± 0.137	BDL-0.696	20.0	1	1.96	0.00
Lambda cyhalothrin	0.701 ± 0.052	BDL-0.765	–	2	3.92	–
Acephate	–	BDL	–	0	0	–
Chlorpyriphos	–	BDL	5.0	0	0	–
Dimethoate	4.793 ± 1.951	BDL-8.667	–	3	5.88	–
Ethion	–	BDL	5.0	0	0	–
Malathion	8.976 ± 1.278	BDL-11.077	5.0	3	5.88	5.88
Monocrotophos	–	BDL	–	0	0	–
Overall mean	–	BDL-11.077		25	49.02	3.00

MRL, maximum residue limit

observed that only 5.88% samples had residue above the prescribed limits.

It is difficult to compare our results with those of other monitoring programmes from other countries as well as with in country, because there are only a few of them published and the range of pesticides considered is different. In India, all the honey samples collected during 1993–1995 were found to contain the residues of organochlorine and organophosphorus pesticides. Besides this carbamate pesticide residues too were found in 55% honey samples. Similar findings were presented in the recent publications (Chawla and Goyal 1988; Anju et al. 1997; Karak et al. 1999; Sarfraz Khan et al. 2004).

Compared to above studies it was observed that honey from Himachal Pradesh had low amount of pesticide residues. However pesticide usage should be managed so that every chance of rejection of honey consignment on the basis of pesticide residues and danger to consumer health may be eliminated.

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