Bull. Environ. Contam. Toxicol. (2005) 74:342–349

DOI: 10.1007/s00128-004-0590-y

Bulletin of Environmental Contamination and Toxicology

Multiresidue Analysis of Pesticides in Animal Feed Concentrate

V. Sharma,[1] B. K. Wadhwa,[1] H. J. Stan[2]

[1] Dairy Chemistry Division, National Dairy Research Institute, Karnal-132001, Haryana, India
[2] Institute of Food Chemistry, Technical University of Berlin, Gustav-Meyer-Allee 25, 13355 Berlin, Germany

Received: 12 May 2004/Accepted: 4 November 2004

In India, during the period 1995-2000, the consumption pattern of pesticides have changed, OC decreased from 40 to 14.5 percent, carbamate from 15 to 4.5 percent and synthetic pyrethroids 10 to 5 percent. There was a sharp increase in the consumption of OP from 30 to 74 percent. Out of 57 insecticides registered for use in agriculture, 8 OP pesticides (monocrotophos, malathion, methyl parathion, phosphamidon, phorate, quinalphos, dimethoate, chlorpyriphos), 1 OCm (carbaryl) and 1 OC (endosulfan) account for 80 percent of the total insecticides used in India (Agnihotri 2000). The major source of contamination of dairy milk by pesticide residues is through contaminated feed. The earlier reports have indicated high levels of DDT and BHC in milk (Kalra and Chawla 1983, SRS Report 1996, Kang et al. 2002). One recent report has also indicated the contamination of organophosphate pesticide residues along with organochloro pesticide residues in animal feed concentrate samples collected from Ludhiana district of Punjab, India (Battu et al. 1996).

The present study was undertaken to evaluate the magnitude of contamination of pesticide residues including organochloro, organophosphate and carbamate in animal feed concentrate samples collected from Karnal (Haryana), India, in light of changed consumption pattern of pesticides in India.

MATERIAL AND METHODS

A total of fifteen animal feed concentrate samples were collected from Karnal (Haryana), India. VAN-MIX consisting of 60 pesticides as listed in Table 6 was prepared for analysis of animal feed samples. All analytical standards were supplied by Dr. Ehrenstorfer, Augsburg, Germany, Promochem, Wesel, Germany and Riedel-De Haen, Seelze, Germany. All solvents, viz., acetonitrile, ethyl acetate, cyclohexane, toluene, n-hexane were suprasolve products from Merck, Darmstadt, Germany. Sodium sulphate, Anhydrous GR grade, Merck, Germany, was heated at 130°C overnight before use. Sodium chloride used was from GR grade, Merck, Germany. Milli-Q reagent grade water system was used throughout analysis. Preparation of pesticide reference standards was carried out on a microbalance (1872 MP 8, Sartorious, Gottingen, Germany), which was

Correspondence to: V. Sharma, Stdelheimer Straße 57, 81549 Munich, Germany

connected to a personal computer running the dedicated windows based software "BALANCE". Pesticides stock solutions were prepared by dissolving pesticide standards in toluene at 1 mg/ml. Working solutions were prepared by mixing and dissolving stock solutions by means of special displacement micropipettes (transferpettors, Brand, Wertheim, Germany). All solutions were registered in the files of the BALANCE program allowing a permanent check of age and quality of working standards, and stock solutions. An ESGE homogenizer (Model SG 2000, Switzerland) was used for sample preparation. Rotary evaporator RV 05 (Janke and Kunkel KG, Germany) equipped with a HWR water bath (DAGLEF PATZ KG) set at 35 to 40°C and a diaphram vacuum pump MZ 2C (vaccubrand) were used to concentrate the organic solvents. 595½, folded filters; ϕ 150 mm from Schleicher and Schuell GmbH, Germany were used for filtration during preparation of sample isolates.

Method of Specht et al. (1995) was used for isolation of multiresidue of pesticides from animal feed samples. Aldrin at level of 100 ppb was used as internal standard. Isolate obtained after complete drying of liquid – liquid partitioning extract was dissolved in 2 ml of ethyl acetate : cyclohexane (1:1). One ml of this extract was used for Gel Permeation Chromatography (GPC). A GPC system consisting of an isocratic HPLC pump, a sample loop of 5 ml, a glass column 470 x 20 mm I.D was used for clean up. GPC glass column packed with Bio-Beads SX-3 and provided with a water jacket to maintain constant temperature, a three way valve with time or sensor switch and two volumetric funnels for fraction collection was used. Ethyl acetate : cyclohexane (1:1, v/v) at a flow rate of 2.5 ml/min was used as eluant. The system was a homemade semiautomatic device. The injection was made manually by filling the extract into the sample loop of 5 ml with a syringe. The GPC was started after resetting all central parameters manually. The eluant was directed into the first volumetric funnel until the liquid surface reaches the sensor; the three-way valve was then automatically switched to transfer the effluent to the sample funnel. The run was finished when the liquid surface reaches the sensor in the sample funnel and the GPC system was ready for next run. The first eluate (100 ml) was discarded, the second eluate (100 ml) containing pesticides was collected and evaporated to dryness and dissolved in 1 ml toluene (Stan 2000). Samples were analysed on GC (ECD and NPD) system and GC-MS. One µl was injection volume for GC and 2 µl was the injection volume for GC-MS system. Recovery experiments were performed in triplicate at 0.1 ppm level for VAN-MIX pesticides.

Gas chromatograph used was from Hewlett-Packard (HP) Model 5890 A series, equipped with 7673A auto sampler, and HP ECD and NPD systems. A hot splitless injector with a 25m x 0.32mm x 0.25µm Permabond OV-17 column was used. Helium of 99.999 percent purity was used as carrier gas. A split ratio of 2:1 for the effluent from the analytical column was obtained by varying lengths of the splitting tubes, 10 cm to the NPD system and 20 cm to ECD system to address the different response sensitivities of the two detectors. Analogue signals obtained from ECD and NPD were recorded in parallel, digitized and transmitted to a personal computer (80486) running under MS Windows-95 and processed with

Table 1.GC parameters for animal feed concentrate.

Injector temperature	210°C
Initial temperature	100°C
Initial time	1 min
Rate	10°C/min
Final temperature	140°C
Final time	0 min
Rate A	3°C/min
Final temperature A	170°C
Final time A	0 min
Rate B	10°C/min
Final temperature B	280°C
Final time B	19 min
ECD temperature	300°C
Range ECD	2
NPD temperature	280°C
Range NPD	0

PE Nelson Analytical Chromatography Software Turbochrom V 4.1 (Perkin Elmer). Table 1 illustrates the analytical parameters of GC (ECD, NPD) system used in the analysis of animal feed concentrate samples.

Gas chromatography – Mass spectrometry used was Finnigan, Magnum; Auto sampler 8200 with HP Chemstation Chromatography software. The column was DB-5 with the dimensions, 30m x 0.32mm x 0.25µm. The mode of injection was hot splitless with programmed temperature vaporization (PTV) injection. The initial temperature of 100°C was kept for 5 sec and then increased to 280°C/20 sec at a rate of 12°C/sec. Finally, the temperature was increased to 300°C/120 sec at a rate of 12°C/sec.

MS measurements were performed with Electron Impact Ionization (EI) at 70 eV. Analysis was done in full scan mode. MS tuning was performed weekly by using the auto tuning macro. MS calibration was done automatically using perfluorotributylamine (PFTBA) as a reference compound. The quality of the system was checked daily with standard solutions and the same special mixture (EINSTIZ Mix) used for ECD / NPD quality check containing aldrin, chlorthion and captan each at 1 ng/µl in toluene. The mixture was also used to check the retention times and to adjust the carrier gas head pressure such that aldrin appears exactly at 12.4 min. Besides retention time; three ions (261, 263, 293) of aldrin were checked for quality of the system. Tables 2 and 3 give the GC-temperature programming and GC-MS acquisition method for EINSTIZ mix. GC-temperature programming and GC-MS acquisition method for sample isolates have been given in Tables 4 and 5, respectively. All chromatograms obtained in pesticide residue screening analysis in full scan mode were evaluated after each run with the Macro

Table 2. GC method for EINSTIZ mix on GC-MS.

Segment	Temperature	Rate	Time	Total
1	90	0.0	1.00	1.00
2	150	30.0	2.00	3.00
3	150	0.0	2.00	5.00
4	193	3.0	14.33	19.33

Table 3. Acquisition method for EINSTIZ mix on GC-MS

Mass range	50 to 506 m/z
Scan time (1 μ scans)	0.310 sec, 3.22 scans/sec
Segment length	19.00 min
Fil / mul delay	2.10 min
Peak threshold	1 counts
Background mass	49 M/z
Method end time	19.00 min
Segment time	0.00 to 19.00

Table 4. GC method for sample isolates on GC-MS

Segment	Temperature	Rate	Time	Total
1	90	0.0	1.00	1.00
2	150	30.0	2.00	3.00
3	150	0.0	2.00	5.00
4	205	3.0	18.33	23.33
5	260	10.0	5.50	28.83
6	260	0.0	15.50	44.33

Table 5. Acquisition method for sample isolates on GC-MS

Mass range	50 to 506 m/z
Scan time (1 μ scans)	0.310 sec, 3.22 scans/sec
Segment length	43.50 min
Fil / mul delay	2.10 min
Peak threshold	1 counts
Background mass	49 M/z
Method end time	43.50 min
Segment time	0.00 to 19.00

program Aupest. The proposals of potential positive pesticide results made by Aupest were checked manually using the interactive capabilities of the program. Aupest (Automated residue analysis on pesticides) is a macro program developed in working group of Professor H.J. Stan, Technical University of Berlin, Germany, for automated evaluation which runs with HP Chemstation Software together with the library PEST.L containing more than 400 active ingredients and also metabolites, environmental contaminants and derivatives of pesticides otherwise not amenable to GC. All target compounds are linked to their retention times measured under fixed conditions. Aupest runs automatically after each full scan GC-MS run and creates a number of reports. The analyst receives all the results in tables together with the raw data, which may simultaneously be accessed in the standalone data analysis of the HP Chemstation after a sample sequence has finished.

RESULTS AND DISCUSSION

Table 6 lists the retention times of VAN-MIX pesticides on GC (ECD and NPD) and on GC-MS by using temperature programming described in Table 1 and 4, respectively and also the results of recovery experiments performed in triplicate at 0.1 ppm for VAN-MIX with the method used.

Table 6. Retention times and recoveries of VAN-MIX pesticides.

Pesticide	Detector		Retention Time		% Recovery
	ECD	NPD	GC	GC-MS	Average ± S.E.
Methamidophos		*	7.80	2.45	58.83 ± 3.04
Dichlorvos	*	*	6.55	2.46	64.62 ± 2.43
Acephate		*	13.25	3.60	79.22 ± 2.54
Propoxur		<	16.51	5.67	81.13 ± 3.16
Bendiocarb		<	18.89	6.17	76.29 ± 2.89
Phorate	<<	*	16.97	6.90	72.07 ± 2.38
Monocrotophos	<<	*	19.59	6.27	86.25 ± 2.80
Dimethoate	*	*	20.12	7.40	71.43 ± 2.55
Carbofuran		<<	19.99	7.47	76.12 ± 2.44
HCH-Gamma	*		19.19	8.00	66.55 ± 2.31
Diazinon	<	*	18.90	9.06	71.22 ± 3.11
Disulfoton	<	*	19.61	8.87	28.85 ± 2.98
Pirimicarb		*	21.00	10.08	73.47 ± 3.50
Phosphamidon I	<	<	20.25	8.80	68.01 ± 5.49
Parathion-methyl	*	*	21.67	10.76	80.04 ± 4.63
Carbaryl	<<	<	22.35	10.97	98.52 ± 4.68
Fenitrothion	*	*	22.30	12.40	51.85 ± 7.93
Methiocarb	<<	*	22.35	12.53	71.45 ± 3.99
Malathion	*	*	22.25	12.88	66.44 ± 3.27
Aldrin	*		21.00	12.40	86.94 ± 3.99
Fenthion	<<	*	22.78	13.09	77.50 ± 3.49
Chlorpyrifos	*	*	22.09	13.23	84.57 ± 3.80

Table 6. continued

Parathion	*	*	22.42	13.40	79.24 ± 4.94
Fenson	*		23.49	13.45	72.99 ± 3.97
Bromophos methyl	*	*	22.72	14.49	102.21 ± 6.06
Mecarbam	*	*	23.50	15.63	69.10 ± 3.35
Captan	*	<<	24.42	14.80	62.88 ± 2.84
Folpet	*	<	24.58	15.17	82.11 ± 4.62
Chlorfenvinfos	*	*	23.33	15.50	76.07 ± 5.61
Methidathion	*	*	24.74	15.93	66.37 ± 5.05
DDE-o,p'	*		23.51	16.15	83.63 ± 5.78
Endosulfan-alpha	*		23.54	16.20	80.50 ± 4.21
DDE-p,p'	*		24.11	17.93	105.28 ± 3.61
Dieldrin	*		24.30	17.54	70.77 ± 4.87
DDD-p,p'	*		25.40	22.25	80.65 ± 4.02
Endosulfan-beta	*		25.63	19.19	82.33 ± 4.75
Ethion	*	*	25.62	20.84	74.45 ± 4.14
DDT-p,p'	*		25.40	22.25	79.55 ± 5.54
Endosulfan sulfate	*		20.68	21.65	69.37 ± 4.01
Propargite	<		26.10	23.80	–
Phosmet	*	*	29.40	24.76	74.05 ± 3.03
Phosalone	<	<	29.13	26.11	81.49 ± 4.71
Amitraz		*	28.15	26.53	–
Azinophos-ethyl	<	*	31.81	27.19	69.73 ± 4.12
Bitertanol		*	30.65	27.89	67.27 ± 3.70
Bitertanol		<	30.88	28.04	
Cyfluthrin	<	<	31.00	28.94	
Cyfluthrin	<	<	31.15	29.09	
Cyfluthrin	<	<	31.26	29.19	75.64 ± 3.11
Cyfluthrin	<	<	31.40	29.26	
Cypermethrin I	*	*	32.02	29.36	
Cypermethrin II	*	*	32.27	29.53	69.03 ± 1.88
Cypermethrin III	*	*	32.53	29.64	
Cypermethrin IV	*	*	32.60	29.70	
Fenvalerate I	*	*	35.49	30.87	77.29 ± 4.06
Fenvalerate II	*	*	36.36	31.29	
Fluvalinate	*	*	33.80	31.39	80.47 ± 2.32
Fluvalinate	*	*	34.10	31.56	
Deltamethrin	<	<	39.90	32.48	82.65 ± 3.77

Response to ECD and NPD : * Good; < Weak; << Very weak

Out of 60 pesticides in VAN-MIX, two pesticides, viz., propargite and amitraz were not recovered. Recovery of methamidophos, fenitrothion, disulfoton and acephate was poor. Recovery of rest of the pesticides was satisfactory and was in the range of 63 to 105 percent.

GC-MS was used both for qualitative, i.e., confirmation of presence of pesticide residue and quantitative purposes. Some of pesticides like DDT isomers and

metabolites, endosulphan (α, β, sulphate) were quantified on GC (ECD and NPD) because these pesticide residues show poor response on MS detector. Limit of detection on GC-MS and GC (ECD and NPD) were 0.001 and 0.0001 ppm, respectively. GC-MS confirmation was done by 'Aupest'. First identification was done with retention time of the pesticide residue, which appeared on a separate window as Aupest report. Peak of identified residue was zoomed to see the mass spectra of the residue. A mass spectrum of sample peak was overlapped with standard peak in two windows. Confirmation of the pesticide residue appeared on the side of the window. Finally, confirmation was done with Aupest level-II, which used reconstructed ion chromatograms (RICs) of the identified pesticide residues.

Out of 60 pesticides selected for multiresidue analysis only detected residues of phorate, monocrotophos, dimethoate, diazinon, carbaryl, chlorpyriphos and p,p'DDT have been listed in Table 8.

Table 8. levels (μg/g fresh basis) of multiresidue of pesticides in animal feed concentrate samples.

Total Samples	Samples contaminated	Pesticide	Average	Range
15	3	Phorate	0.007 ± 0.003	ND – 0.041
	3	Monocrotophos	0.008 ± 0.003	ND – 0.042
	4	Dimethoate	0.015 ± 0.006	ND – 0.061
	4	Diazinon	0.011 ± 0.006	ND – 0.089
	1	Carbaryl	0.002 ± 0.002	ND – 0.031
	2	Chlorpyriphos	0.009 ± 0.004	ND – 0.042
	2	p,p'-DDT	0.007 ± 0.005	ND – 0.078

Among the organocarbamate pesticide residues carbaryl was detected in one sample only. Among the organochloro pesticide residues only p,p'-DDT was detected in two samples. The results obtained for DDT are in accordance with the earlier reports. Kalra and Chawla (1983) revealed contamination of animal feed with organochloro pesticide residues, viz., DDT and BHC. The levels of contamination in animal feed like oil seed cake, groundnut husk, wheat straw and fodder were 0.107, 0.228, 0.37 and 0.05 ppm, respectively, for DDT residues. Further, levels of DDT were 0.005 – 49.1 ppm, and of BHC were 7.6 – 119.7 ppm in commercial feed concentrate (SRS Report 1996). Monitoring of 105 samples of different feeds from Punjab revealed 100 and 80 percent contamination of residues of BHC and DDT, respectively. Kang et al. (2002) evaluated samples of animal feed concentrate and green fodder for BHC isomers, DDT isomers and metabolites, endosulphan, dicofol, monocrotophos, chlorpyriphos, methyl parathion, quinalphos, malathion and triazophos. For levels of organochlorine pesticide residues result showed a substantial decline with respect to β-BHC and total DDT as compared to earlier report of Battu et al. (1996). Endosulphan and dicofol residues were detected in one and two samples, respectively. However,

among the organophosphates, only malathion residues were detected in eight samples with their levels ranging from BDL – 4.35 mg/kg.

The present study reports DDT in only two feed concentrate samples. This decline in levels could be attributed to ban of DDT in the agriculture sector since 1984. However, presence of organophosphate pesticide residues in animal feed concentrate in the present study could be due to major consumption of organophosphates in agriculture sector. Our findings are in accordance with the recent observations made by Kang et al. 2002. The results are also in accordance with major pesticides used in India (Agnihotri 2000).

Acknowledgements: I thank Prof. H.J.Stan for providing me all the facilities and guidance for this work. Also, I am thankful to DAAD (German Academic Exchange Service) Scholarship for giving me opportunity to carry out extension of Ph.D. work at Technical University-Berlin, Germany.

REFERENCES

Agnihotri NP (2000) Pesticide consumption in agriculture in India – an update. Pesticide Res J 12: 150-155

Battu RS, Singh B, Chahal KK, Kalra RL (1996) Contamination of animal feed with residues of HCH and DDT. Pesticide Res J 8: 172-175.

Kalra RL, Chawala RP (1983) Studies on pesticide residues and monitoring of pesticidal pollution. Final Technical Report, PL-480 Project, Punjab Agricultural University, Ludhiana.

Kang BK, Singh B, Chahal KK, Battu RS (2002) Contamination of feed concentrate and green fodder with pesticide residues. Pesticide Res J 14: 308-312.

NDRI / SRS Report. (1996) Pesticide residues in Indian milk and milk products and their origin. Final Report on Research Scheme, Department of Dairy Chemistry, SRS of NDRI, Bangalore.

Specht W, Pelz S, Gilsbach W (1995) Gas chromatographic determination of pesticide residues after clean-up gel permeation chromatography and mini silica gel column chromatograph. Fresenius J Anal Chem 353: 183-190.

Stan HJ (2000) Pesticide residue analysis in foodstuffs applying capillary gas Chromatography with mass spectrometric detection. State-of-the-art use of modified DFG-multimethod S19 and automated data evaluation. J Chromatog 892: 347-377