African Journal of Environmental Science and Technology Vol. 4(10), pp. 666-684, October 2010
Available online at http://www.academicjournals.org/AJEST
ISSN 1991-637X ©2010 Academic Journals

Full Length Research Paper

Determination of pesticide residues in blood samples of villagers involved in pesticide application at District Vehari (Punjab), Pakistan

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Accepted July 13, 2010

Blood samples were collected from field workers involved in pesticide spraying activities at three different farms in Tahsil Mailsi, District Vehari (Punjab), Pakistan. Twenty seven villagers (including three controls), ranging from 16 to 50 years of age and one to nine years of pesticide application experience were tested. The blood samples were analyzed for 383 different pesticides using Gas Chromatograph Mass Spectrometer (GC-MS) multi residue analytical technique. Only chlorpyrifos (0.009 mg/l) and pyributicarb (0.001 mg/l) were detected in the blood samples.

Key words: Multi-pesticide residues, blood sample, GC-MS.

INTRODUCTION

Pesticide is a general term for substances, which are used to poison pests (weeds, insects, molds, rodents etc.). The pesticides, most acutely dangerous to humans are insecticides and rodenticides (Mathur et al., 2005). Synthetic pesticides have been popular with farmers, because of their widespread availability, simplicity in application, efficacy and economic returns. MINFAL (2004) reported that the climate condition in Pakistan favors the population of insect pests like white fly, jassids, aphids, boll worm and fruit flies, and pesticides ranging from 24,868 MT in 1994 to 112,928 MT in 2004 have been used to control them. They also have huge environmental costs; along with this, some cases of intentional or unintentional pesticide poisoning have been reported from time to time among unseen and actual farmers.

Pesticides, widespread in the agriculture sector, are the most economical approach used to control insects and pests, even though they are the major contaminants of our environment and are highly toxic to non-target organisms. The spray-workers, during the activity of spraying crops, are directly exposed to pesticides while mixing, handling and spraying. Also, they are exposed to pesticides through contaminated soil, air, drinking water, eating food and smoking at work places. Ultimately, these are absorbed by inhalation, ingestion and dermal contact (Vega, 1994). The residue concentrations of these compounds in the exposed spray-workers can lead to a variety of metabolic and systemic dysfunctions, and in some cases outright disease states.

Therefore, the tremendous usage of pesticides has promoted toxicological studies in spraying communities and as a result, the common mode of action for the major pesticide products is to disrupt the neurological function (Brown et al., 1989; Karalliede et al., 1999). In addition to being neurotoxic, these compounds are profoundly injurious to the immune and endocrine systems as well (Chambers et al., 1992; Arlien-Soberg, 1992; Luster et al., 1993). Such ill health effects are not limited only to those systems, but can cause a variety of dermatological, gastrointestinal, genito-urinary, respiratory, musculo-skeletal and cardiological problems (Hueser, 1992; Vial et al., 1996).

Globally, various products of different pesticide groups have been investigated by environmental toxicologists for their toxic end points; as reports indicated the accumulation of DDT, BHC and endosulfan which have been implicated in the pathogenesis of cardiovascular disorders, hypertension and other health related problems

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(Chandra et al., 1992). The residue concentrations of some organochlorine and organophos-phorus pesticides were also detected in blood samples of school children (Mohammed et al., 2001), which prompt the adult studies in the directly exposed spray workers. Monocrotophos, a product of organopho-sphates group, was massively used in the agriculture sector and reported for environ-mental persistence (Burkhard et al., 1994) with half-life around seven days in soil exposed to natural sunlight (Smith, 1993; Meister, 1992; U.S EPA, 1985). Being a cholinesterase inhibitor, it was recorded as a potent neurotoxic agent (Qadri et al., 1994; Rao et al., 2001; Rao, 2004).

An April 2005 study published in the Pakistan Medical Sciences Journal reported that out of a total of 578 patients admitted to Nishtar hospital between 1996 and 2000 with chemical poisoning; about 64% were victims of organic pesticide poisoning, of which around 73% were males and 27% were females. The research worldwide has shown that pesticides are responsible for various incidences of cancer and other diseases in humans. As the villagers involved in spray application have maximum chances to receive pesticides from dermal contact, so the analysis of blood will provide evidence of exposure of the body to pesticides and give an indication of the body burden of the pesticide residues (IRIN Report, 2005).

Monitoring OC concentration in blood is most appropriate because these pesticides are lipophilic in nature. Similarly, monitoring OP concentrations in blood or blood products (serum, plasma) offers several advan-tages. The parent compounds can be monitored directly in blood instead of their metabolites, which are usually measured in urine. Blood measurements provide an estimation of the dose available for the target site, allowing for prediction of dose-response relationships. Furthermore, because blood is a regulated fluid (the volume does not vary substantially with water intake or other factors), the blood concentrations of toxicants measured at a specific time interval after exposure will remain the same as long as the absorbed amounts are constant; therefore, no corrections for dilution are necessary (Wessels et al., 2003). However, the present study will also help to estimate the penetration ability of pesticide due to spraying activities.

MATERIALS AND METHODS

Study area

The experiment was conducted in field and laboratory. Samples of blood were collected from field workers involved in spraying activities at three different farms in Tahsil Mailsi, District Vehari (Punjab), Pakistan. For this venture, blood (10 ml) of 27 villagers (including three controls), ranging from 16 to 50 years of age and one to nine years of pesticide application experience (Annexure I), was collected. Three villagers were selected from each of the three farms in three different villages. Blood samples were collected in residue free heparinised glass vials with the help of sterilized syringe. Blood samples were transported in dry ice to the laboratory and stored at – 20°C until they were analyzed. Samples were analyzed in the Pesticide Residue Testing Lab. in Kala Shah Kako Lahore, Pakistan.

Solvents and glassware

All the solvents, that is, acetone, diethyl ether and hexane (HPLC grade), used for the analysis were purchased from E-Merck. Organic solvents were glass distilled and checked for any pesticide contamination. All glassware were washed with detergent, rinsed with water, dipped in chromic acid for 24 h and finally rinsed with distilled water and then hexane.

Chemicals

Pesticide reference standards were purchased from Sigma Chemicals and about 383 pesticides can be detected in a single assay (Table 1).

Sample extraction and clean up

Extraction

Extraction was based on the method followed by Agarwal et al. (1976) with some modifications. Blood (5 ml) was diluted with 25 ml distilled water and a 2 ml of saturated brine solution was added, then it was transferred to a 125 ml capacity separatory funnel. It was extracted with hexane: acetone (1:1) (20 ml) (thrice), by shaking the separatory funnel vigorously for 2 - 3 min, thereby releasing the pressure intermittently. As a result, the layers were allowed to separate. The three combined extracts were passed through anhydrous sodium sulfate and concentrated to about 1 - 2 ml using a rotary vacuum evaporator. Consequently, the whole blood was used.

Clean up

Clean up was done by USEPA method 3620B (Florisil clean up by column chromatography). Florisil was activated at 130°C overnight and cooled in a dessicator before use. The weight of florisil taken was predetermined by calibration using lauric acid and 1 g of florisil was packed in the 20 cm length and 12 mm ID glass chroma-tographic column. Anhydrous sodium sulfate was added to the top of the florisil column (0.5 cm) and the column was pre-eluted with hexane, and was then discarded. The extract was transferred to the column and eluted with hexane (10 ml), 6% diethyl ether in hexane (10 ml), 15% diethyl ether in hexane (10 ml), 50% diethyl ether in hexane (10 ml) and finally with diethyl ether (10 ml). As a result, eluent was collected and evaporated to dryness. The final samples were prepared in 2 ml hexane (HPLC grade) and were then analyzed.

Sample analysis

Calibration of GC-MS system: GC system was calibrated using external standard technique.

Stock standard solution (1000 mg/l)

Stock solution was prepared by weighing appropriate amounts of active ingredients in a brown bottle with a teflon-lined screw cap and dissolving the weighed standard in HPLC grade hexane. Stock standard solution was used to prepare primary dilution standards.

Table 1. A total of 383 pesticide, retention times, quantifying ions, qualifying ions, relative abundance, limit of quantitation (LOQ) and correlation coefficient of pesticides by gas chromatography-mass spectrometry (GC-MS) (Pang et al., 2006).

Number	Pesticide	Retention time(min)	Quantifying ion*		Qualifying ion 1*		Qualifying ion 2*		Qualifying ion 3*		LOQ (mgkg^{-1})	Correlation coefficient(r)
SIM-AISTD	Heptachlor-epoxide	22.10	353	(100)	355	(79)	351	(52)				
1	Allidochlor	8.78	138	(100)	158	(10)	173	(15)			0.0250	0.9986
2	Dichlormid	9.74	172	(100)	166	(41)	124	(79)			0.0125	0.9981
3	Etridiazol	10.42	211	(100)	183	(73)	140	(19)			0.0375	0.9945
4	Chlormephos	10.53	121	(100)	234	(70)	154	(70)			0.0250	0.9928
5	Propham	11.36	179	(100)	137	(66)	120	(51)			0.0125	0.9975
6	Cycloate	13.56	154	(100)	186	(5)	215	(12)			0.0125	0.9986
7	Diphenylamine	14.55	169	(100)	168	(58)	167	(29)			0.0125	0.9975
8	Chlordimeform	14.93	196	(100)	198	(30)	195	(18)	183	(23)	0.0250	0.9966
9	Ethalfluralin	15.00	276	(100)	316	(81)	292	(42)			0.0500	0.9926
10	Phorate	15.46	260	(100)	121	(160)	231	(56)	153	(3)	0.0125	0.9969
11	Thiometon	16.20	88	(100)	125	(55)	246	(9)			0.0125	0.9967
12	Quintozene	16.75	295	(100)	237	(159)	249	(114)			0.0250	0.9958
13	Atrazine-desethyl	16.76	172	(100)	187	(32)	145	(17)			0.0125	0.9956
14	Clomazone	17.00	204	(100)	138	(4)	205	(13)			0.0125	0.9976
15	Diazinon	17.14	304	(100)	179	(192)	137	(172)			0.0125	0.9980
16	Fonofos	17.31	246	(100)	137	(141)	174	(15)	202	(6)	0.0125	0.9975
17	Etrimfos	17.92	292	(100)	181	(40)	277	(31)			0.0125	0.9976
18	Simazine	17.85	201	(100)	186	(62)	173	(42)			0.0125	0.9965
19	Propetamphos	17.97	138	(100)	194	(49)	236	(30)			0.0125	0.9968
20	Secbumeton	18.36	196	(100)	210	(38)	225	(39)			0.0125	0.9952
21	Dichlofenthion	18.80	279	(100)	223	(78)	251	(38)			0.0125	0.9986
22	Pronamide	18.72	173	(100)	175	(62)	255	(22)			0.0125	0.9905
23	Mexacarbate	18.83	165	(100)	150	(66)	222	(27)			0.0375	0.9924
24	Dimethoate	19.52	125	(100)	143	(21)	229	(19)			0.0500	0.9931
25	Aldrin	19.67	263	(100)	265	(65)	293	(40)	329	(8)	0.0250	0.9990
26	Dinitramine	19.35	305	(100)	307	(38)	261	(29)			0.0500	0.9956
27	Ronnel	19.80	285	(100)	287	(67)	125	(32)			0.0250	0.9978
28	Prometryne	20.13	241	(100)	184	(78)	226	(60)			0.0125	0.9961
29	Cyprazine	20.18	212	(100)	227	(58)	170	(29)			0.0125	0.9978
30	Chlorothalonil	20.22	266	(100)	264	(72)	268	(49)			0.0250	0.9926
31	Vinclozolin	20.29	285	(100)	212	(109)	198	(96)			0.0125	0.9973
32	Beta-HCH	20.31	219	(100)	217	(78)	181	(94)	254	(12)	0.0125	0.9992
33	Metalaxyl	20.67	206	(100)	249	(53)	234	(38)			0.0375	0.9982

Table 1. Continued.

34	Chlorpyrifos (-ethyl)	20.96	314	(100)	258	(57)	286	(42)			0.0125	0.9985
35	Methyl-parathion	20.82	263	(100)	233	(66)	246	(8)	200	(6)	0.0500	0.9892
36	Anthraquinone	21.49	208	(100)	180	(84)	152	(69)			0.0125	0.9862
37	Delta-HCH	21.16	219	(100)	217	(80)	181	(99)	254	(10)	0.0250	0.9986
38	Fenthion	21.53	278	(100)	169	(16)	153	(9)			0.0125	0.9973
39	Malathion	21.54	173	(100)	158	(36)	143	(15)			0.0500	0.9974
40	Fenitrothion	21.62	277	(100)	260	(52)	247	(60)			0.0250	0.9957
41	Paraoxon-ethyl	21.57	275	(100)	220	(60)	247	(58)			0.0500	0.9892
42	Triadimefon	22.22	208	(100)	210	(50)	181	(74)			0.0250	0.9976
43	Parathion	22.32	291	(100)	186	(23)	235	(35)	263	(11)	0.0500	0.9935
44	Pendimethalin	22.59	252	(100)	220	(22)	162	(12)			0.0500	0.9960
45	Linuron	22.44	61	(100)	248	(30)	160	(12)			0.0500	0.9730
46	Chlorbenside	22.96	268	(100)	270	(41)	143	(11)			0.0250	0.9946
47	Bromophos-ethyl	23.06	359	(100)	303	(77)	357	(74)			0.0125	0.9973
48	Quinalphos	23.10	146	(100)	298	(28)	157	(66)			0.0125	0.9959
49	trans-Chlordane	23.29	373	(100)	375	(96)	377	(51)			0.0125	0.9989
50	Phenthoate	23.30	274	(100)	246	(24)	320	(5)			0.0250	0.9970
51	Metazachlor	23.32	209	(100)	133	(120)	211	(32)			0.0375	0.9984
52	Fenothiocarb	23.79	72	(100)	160	(37)	253	(15)			0.2000	0.9955
53	Prothiophos	24.04	309	(100)	267	(88)	162	(55)			0.0125	0.9957
54	Folpet	24.10	260	(100)	104	(56)	297	(20)			0.0750	0.9919
55	Chlorfurenol	24.15	215	(100)	152	(40)	274	(11)			0.0375	0.9960
56	Dieldrin	24.43	263	(100)	277	(82)	380	(30)	345	(35)	0.0250	0.9994
57	Procymidone	24.36	283	(100)	285	(70)	255	(15)			0.0125	0.9979
58	Methidathion	24.49	145	(100)	157	(2)	302	(4)			0.0250	0.9948
59	Cyanazine	24.94	225	(100)	240	(56)	198	(61)			0.0375	0.9952
60	Napropamide	24.84	271	(100)	128	(111)	171	(34)			0.0375	0.9963
61	Oxadiazone	25.06	175	(100)	258	(62)	302	(37)			0.0125	0.9980
62	Fenamiphos	25.29	303	(100)	154	(56)	288	(31)	217	(22)	0.0375	0.9864
63	Tetrasul	25.85	252	(100)	324	(64)	254	(68)			0.0125	0.9958
64	Aramite	25.60	185	(100)	319	(37)	334	(32)			0.0125	0.9927
65	Bupirimate	26.00	273	(100)	316	(41)	208	(83)			0.0125	0.9954
66	Carboxin	26.25	235	(100)	143	(168)	87	(52)			0.0375	0.9930
67	Flutolanil	26.23	173	(100)	145	(25)	323	(14)			0.0125	0.9951
68	4,40-DDD	26.59	235	(100)	237	(64)	199	(12)	165	(46)	0.0125	0.9930
69	Ethion	26.69	231	(100)	384	(13)	199	(9)			0.0250	0.9944

Table 1. Continued.

70	Sulprofos	26.87		322	(100)	156	(62)	280	(11)			0.0250	0.9959
71	Etaconazole	26.81		245	(100)	173	(85)	247	(65)			0.0375	0.9948
72	Myclobutanil	27.19		179	(100)	288	(14)	150	(45)			0.0125	0.9958
73	Diclofop-methyl	28.08		253	(100)	281	(50)	342	(82)			0.0125	0.9958
74	Propiconazole	28.15		259	(100)	173	(97)	261	(65)			0.0375	0.9942
75	Fensulfothin	27.94		292	(100)	308	(22)	293	(73)			0.0250	0.9900
76	Bifenthrin	28.57		181	(100)	166	(25)	165	(23)			0.0125	0.9936
77	Carbosulfan	28.67		160	(100)	118	(74)	323	(14)			0.0250	0.9998
78	Mirex	28.72		272	(100)	237	(49)	274	(80)			0.0125	0.9974
79	Benodanil	29.14		231	(100)	323	(38)	203	(22)			0.0375	0.9899
80	Nuarimol	28.90		314	(100)	235	(155)	203	(108)			0.0250	0.9962
81	Methoxychlor	29.38		227	(100)	228	(16)	212	(4)			0.0125	0.9921
82	Oxadixyl	29.50		163	(100)	233	(18)	278	(11)			0.0125	0.9897
83	Tetramethirn	29.59		164	(100)	135	(3)	232	(1)			0.0250	0.9928
84	Tebuconazole	29.51		250	(100)	163	(55)	252	(36)			0.0375	0.9941
85	Norflurazon	29.99		303	(100)	145	(101)	102	(47)			0.0125	0.9879
86	Pyridaphenthion	30.17		340	(100)	199	(48)	188	(51)			0.0125	0.9841
87	Phosmet	30.46		160	(100)	161	(11)	317	(4)			0.0250	0.9861
88	Tetradifon	30.70		227	(100)	356	(70)	159	(196)			0.0125	0.9966
89	Oxycarboxin	31.00		175	(100)	267	(52)	250	(3)			0.0750	0.9850
90	cis-Permethrin	31.42		183	(100)	184	(15)	255	(2)			0.0125	0.9935
91	trans-Permethrin	31.68		183	(100)	184	(15)	255	(2)			0.0125	0.9911
92	Pyrazophos	31.60		221	(100)	232	(35)	373	(19)			0.0250	0.9904
93	Cypermethrin	33.19	33.38	181	(100)	152	(23)	180	(16)			0.0375	0.9919
		33.46	33.56										
94	Fenvalerate	34.45	34.79	167	(100)	225	(53)	419	(37)	181	(41)	0.0500	0.9928
95	Deltamethrin	35.77		181	(100)	172	(25)	174	(25)			0.0750	0.9921
SIM-B													
96	EPTC	8.54		128	(100)	189	(30)	132	(32)			0.0375	0.9993
97	Butylate	9.49		156	(100)	146	(115)	217	(27)			0.0375	0.9994
98	Dichlobenil	9.75		171	(100)	173	(68)	136	(15)			0.0050	0.9998
99	Pebulate	10.18		128	(100)	161	(21)	203	(20)			0.0375	0.9994
100	Nitrapyrin	10.89		194	(100)	196	(97)	198	(23)			0.0375	0.9957
101	Mevinphos	11.23		127	(100)	192	(39)	164	(29)			0.0250	0.9957
102	Chloroneb	11.85		191	(100)	193	(67)	206	(66)			0.0125	0.9990

Table 1. Continued.

103	Tecnazene	13.54	261	(100)	203	(135)	215	(113)			0.0500	0.9985
104	Heptenophos	13.78	124	(100)	215	(17)	250	(14)			0.0375	0.9979
105	Hexachlorobenzene	14.69	284	(100)	286	(81)	282	(51)			0.0125	0.9996
106	Ethoprophos	14.40	158	(100)	200	(40)	242	(23)	168	(15)	0.0375	0.9982
107	Propachlor	14.73	120	(100)	176	(45)	211	(11)			0.0375	0.9998
108	Diallate	15.29	234	(100)	236	(37)	128	(38)			0.0250	0.9994
109	Trifluralin	15.23	306	(100)	264	(72)	335	(7)			0.0250	0.9930
110	Chlorpropham	15.49	213	(100)	171	(59)	153	(24)			0.0250	0.9971
111	Sulfotep	15.55	322	(100)	202	(43)	238	(27)	266	(24)	0.0125	0.9991
112	Sulfallate	15.75	188	(100)	116	(7)	148	(4)			0.0250	0.9958
113	Alpha-HCH	16.06	219	(100)	183	(98)	221	(47)	254	(6)	0.0125	0.9994
114	Terbufos	16.83	231	(100)	153	(25)	288	(10)	186	(13)	0.0250	0.9977
115	Terbumeton	17.20	210	(100)	169	(66)	225	(32)			0.0375	0.9983
116	Profluralin	17.36	318	(100)	304	(47)	347	(13)			0.0500	0.9955
117	Dioxathion	17.51	270	(100)	197	(43)	169	(19)			0.0500	0.9993
118	Propazine	17.67	214	(100)	229	(67)	172	(51)			0.0125	0.9988
119	Chlorbufam	17.85	223	(100)	153	(53)	164	(64)			0.0500	0.9921
120	Dicloran	17.89	206	(100)	176	(128)	160	(52)			0.0500	0.9963
121	Terbuthylazine	18.07	214	(100)	229	(33)	173	(35)			0.0125	0.9886
122	Monolinuron	18.15	61	(100)	126	(45)	214	(51)			0.0500	0.9888
123	Flufenoxuron	18.83	305	(100)	126	(67)	307	(32)			0.0375	0.9929
124	Cyanophos	18.73	243	(100)	180	(8)	148	(3)			0.0250	0.9980
125	Chlorpyrifos-methyl	19.38	286	(100)	288	(70)	197	(5)			0.0125	0.9972
126	Desmetryn	19.64	213	(100)	198	(60)	171	(30)			0.0125	0.9964
127	Dimethachlor	19.80	134	(100)	197	(47)	210	(16)			0.0375	0.9989
128	Alachlor	20.03	188	(100)	237	(35)	269	(15)			0.0375	0.9990
129	Pirimiphos-methyl	20.30	290	(100)	276	(86)	305	(74)			0.0125	0.9977
130	Terbutryn	20.61	226	(100)	241	(64)	185	(73)			0.0250	0.9980
131	Thiobencarb	20.63	100	(100)	257	(25)	259	(9)			0.0250	0.9987
132	Aspon	20.62	211	(100)	253	(52)	378	(14)			0.0250	0.9994
133	Dicofol	21.33	139	(100)	141	(72)	250	(23)	251	(4)	0.0250	0.9980
134	Metolachlor	21.34	238	(100)	162	(159)	240	(33)			0.0125	0.9977
135	Oxy-chlordane	21.63	387	(100)	237	(50)	185	(68)			0.0500	0.9997
136	Pirimiphos-ethyl	21.59	333	(100)	318	(93)	304	(69)			0.0250	0.9980
137	Methoprene	21.71	73	(100)	191	(29)	153	(29)			0.0500	0.9975
138	Bromofos	21.75	331	(100)	329	(75)	213	(7)			0.0250	0.9987

Table 1. Continued.

139	Dichlofluanid	21.68	224	(100)	226	(74)	167	(120)			0.0750	0.9977
140	Ethofumesate	21.84	207	(100)	161	(54)	286	(27)			0.0250	0.9992
141	Isopropalin	22.10	280	(100)	238	(40)	222	(4)			0.0250	0.9982
142	Endosulfan-1	23.10	241	(100)	265	(66)	339	(46)			0.0750	0.9991
143	Propanil	22.68	161	(100)	217	(21)	163	(62)			0.0250	0.9930
144	Isofenphos	22.99	213	(100)	255	(44)	185	(45)			0.0250	0.9982
145	Crufomate	22.93	256	(100)	182	(154)	276	(58)			0.0750	0.9914
146	Chlorfenvinphos	23.19	323	(100)	267	(139)	269	(92)			0.0375	0.9976
147	cis-Chlordane	23.55	373	(100)	375	(96)	377	(51)			0.0250	0.9994
148	Tolylfluanide	23.45	238	(100)	240	(71)	137	(210)			0.0375	0.9981
149	4,4^{0}-DDE	23.92	318	(100)	316	(80)	246	(139)	248	(70)	0.0125	0.9994
150	Butachlor	23.82	176	(100)	160	(75)	188	(46)			0.0250	0.9970
151	Chlozolinate	23.83	259	(100)	188	(83)	331	(91)			0.0250	0.9994
152	Crotoxyphos	23.94	193	(100)	194	(16)	166	(51)			0.0750	0.9904
153	Iodofenphos	24.33	377	(100)	379	(37)	250	(6)			0.0250	0.9940
154	Tetrachlorvinphos	24.36	329	(100)	331	(96)	333	(31)			0.0375	0.9975
155	Chlorbromuron	24.37	61	(100)	294	(17)	292	(13)			0.3000	0.9843
156	Profenofos	24.65	339	(100)	374	(39)	297	(37)			0.0750	0.9969
157	Fluorochloridone	25.14	311	(100)	313	(64)	187	(85)			0.1000	0.9994
158	Buprofezin	24.87	105	(100)	172	(54)	305	(24)			0.0250	0.9987
159	2,40-DDD	24.94	235	(100)	237	(65)	165	(39)	199	(15)	0.0250	0.9997
160	Endrin	25.15	263	(100)	317	(30)	345	(26)			0.1500	0.9982
161	Hexaconazole	24.92	214	(100)	231	(62)	256	(26)			0.1500	0.9989
162	Chlorfenson	25.05	302	(100)	175	(282)	177	(103)			0.0250	0.9980
163	2,40-DDT	25.56	235	(100)	237	(63)	165	(37)	199	(14)	0.0250	0.9978
164	Paclobutrazol	25.21	236	(100)	238	(37)	167	(39)			0.0375	0.9941
165	Methoprotryne	25.63	256	(100)	213	(24)	271	(17)			0.0375	0.9973
166	Erbon	25.68	169	(100)	171	(35)	223	(30)			0.0250	0.9994
167	Chloropropylate	25.85	251	(100)	253	(64)	141	(18)			0.0125	0.9970
168	Flamprop-methyl	25.90	105	(100)	77	(26)	276	(11)			0.0125	0.9983
169	Nitrofen	26.12	283	(100)	253	(90)	202	(48)	139	(15)	0.0750	0.9905
170	Oxyfluorfen	26.13	252	(100)	361	(35)	300	(35)			0.0500	0.9945
171	Chlorthiophos	26.52	325	(100)	360	(52)	297	(54)			0.0375	0.9978
172	Flamprop-isopropyl	26.70	105	(100)	276	(19)	363	(3)			0.0125	0.9974
173	4,40-DDT	27.22	235	(100)	237	(65)	246	(7)	165	(34)	0.0250	0.9971
174	Carbofenothion	27.19	157	(100)	342	(49)	199	(28)			0.0250	0.9955

Table 1. Continued.

175	Benalyxyl	27.54		148	(100)	206	(32)	325	(8)			0.0125	0.9972
176	Edifenphos	27.94		173	(100)	310	(76)	201	(37)			0.0250	0.9912
177	Triazophos	28.23		161	(100)	172	(47)	257	(38)			0.0375	0.9944
178	Cyanofenphos	28.43		157	(100)	169	(56)	303	(20)			0.0125	0.9967
179	Chlorbenside sulfone	28.88		127	(100)	99	(14)	89	(33)			0.0250	0.9966
180	Endosulfan-sulfate	29.05		387	(100)	272	(165)	389	(64)			0.0375	0.9985
181	Bromopropylate	29.30		341	(100)	183	(34)	339	(49)			0.0250	0.9963
182	Benzoylprop-ethyl	29.40		292	(100)	365	(36)	260	(37)			0.0375	0.9983
183	Fenpropathrin	29.56		265	(100)	181	(237)	349	(25)			0.0250	0.9966
184	Captafol	29.96		79	(100)	183	(32)	311	(15)			0.4500	0.9994
185	Leptophos	30.19		377	(100)	375	(73)	379	(28)			0.0250	
186	EPN	30.06		157	(100)	169	(53)	323	(14)			0.0500	0.9913
187	Hexazinone	30.14		171	(100)	252	(3)	128	(12)			0.0375	0.9970
188	Bifenox	30.47		341	(100)	189	(82)	310	(75)			0.0250	
189	Phosalone	31.22		182	(100)	367	(30)	154	(20)			0.0250	0.9955
190	Azinphos-methyl	31.41		160	(100)	132	(71)	77	(58)			0.1500	0.9971
191	Fenarimol	31.65		139	(100)	219	(70)	330	(42)			0.0250	0.9993
192	Azinphos-ethyl	32.01		160	(100)	132	(103)	77	(51)			0.0250	0.9930
193	Prochloraz	33.07		180	(100)	308	(59)	266	(18)			0.0750	0.9919
194	Coumaphos	33.22		362	(100)	226	(56)	364	(39)	334	(15)	0.0750	0.9955
195	Cyfluthrin	32.94	33.12	206	(100)	199	(63)	226	(72)			0.1500	0.9966
196	Fluvalinate	34.94	35.02	250	(100)	252	(38)	181	(18)			0.1500	0.9954
SIM-C													
197	Dichlorvos	7.80		109	(100)	185	(34)	220	(7)			0.0750	0.9990
198	Biphenyl	9.00		154	(100)	153	(40)	152	(27)			0.0125	0.9998
199	Propamocarb	9.58		58	(100)	129	(6)	188	(1)			0.0750	
200	Vernolate	9.82		128	(100)	146	(17)	203	(9)			0.0125	0.9997
201	3,5-Dichloroaniline	11.20		161	(100)	163	(62)	126	(10)			0.0125	0.9905
202	Molinate	11.92		126	(100)	187	(24)	158	(2)			0.0125	0.9996
203	Methacrifos	11.86		125	(100)	208	(74)	240	(44)			0.0125	0.9994
204	2-Phenylphenol	12.47		170	(100)	169	(72)	141	(31)			0.0125	0.9946
205	Cis-1,2,3,6-Tetrahydro-phthalimide	13.39		151	(100)	123	(16)	122	(16)			0.0375	0.9985
206	Fenobucarb	14.60		121	(100)	150	(32)	107	(8)			0.0250	0.9982
207	Benfluralin	15.23		292	(100)	264	(20)	276	(13)			0.0125	0.9888

Table 1. Continued.

208	Hexaflumuron	16.20	176	(100)	279	(28)	277	(43)			0.0750	
209	Prometon	16.66	210	(100)	225	(91)	168	(67)			0.0375	0.9988
210	Triallate	17.12	268	(100)	270	(73)	143	(19)			0.0250	0.9993
211	Pyrimethanil	17.28	198	(100)	199	(45)	200	(5)			0.0125	0.9980
212	Gamma-HCH	17.48	183	(100)	219	(93)	254	(13)	221	(40)	0.0250	0.9998
213	Disulfoton	17.61	88	(100)	274	(15)	186	(18)			0.0125	0.9983
214	Atrizine	17.64	200	(100)	215	(62)	173	(29)			0.0125	0.9991
215	Heptachlor	18.49	272	(100)	237	(40)	337	(27)			0.0375	0.9990
216	Iprobenfos	18.44	204	(100)	246	(18)	288	(17)			0.0375	0.9895
217	Isazofos	18.54	161	(100)	257	(53)	285	(39)	313	(14)	0.0250	0.9994
218	Plifenate	18.87	217	(100)	175	(96)	242	(91)			0.0500	0.9992
219	Fenpropimorph	19.22	128	(100)	303	(5)	129	(9)			0.0125	0.9989
220	Transfluthrin	19.04	163	(100)	165	(23)	335	(7)			0.0125	0.9991
221	Fluchloralin	18.89	306	(100)	326	(87)	264	(54)			0.0500	0.9952
222	Tolclofos-methyl	19.69	265	(100)	267	(36)	250	(10)			0.0125	0.9991
223	Propisochlor	19.89	162	(100)	223	(200)	146	(17)			0.0125	
224	Ametryn	20.11	227	(100)	212	(53)	185	(17)			0.0375	0.9989
225	Simetryn	20.18	213	(100)	170	(26)	198	(16)			0.0250	0.9990
226	Metobromuron	20.07	61	(100)	258	(11)	170	(16)			0.0750	0.9986
227	Metribuzin	20.33	198	(100)	199	(21)	144	(12)			0.0375	0.9986
228	Dimethipin	20.38	118	(100)	210	(26)	103	(20)			0.0375	
229	Epsilon-HCH	20.78	181	(100)	219	(76)	254	(15)	217	(40)	0.0250	
230	Dipropetryn	20.82	255	(100)	240	(42)	222	(20)			0.0125	0.9976
231	Formothion	21.22	170	(100)	224	(97)	257	(63)			0.1000	0.9993
232	Terbacil	21.33	161	(100)	160	(53)	117	(45)			0.0750	0.9983
233	Diethofencarb	21.43	267	(100)	225	(98)	151	(31)			0.0750	0.9977
234	Dimepiperate	22.28	119	(100)	145	(30)	263	(8)			0.5000	1.0000
235	Bioallethrin	22.29	123	(100)	136	(24)	107	(29)			0.0250	0.9941
236	2,40-DDE	22.64	246	(100)	318	(34)	176	(26)	248	(65)	0.0125	0.9993
237	Fenson	22.54	141	(100)	268	(53)	77	(104)			0.2500	1.0000
238	Diphenamid	22.87	167	(100)	239	(30)	165	(43)			0.0125	0.9989
239	Chlorthion	22.86	297	(100)	267	(162)	299	(45)			0.1000	0.9857
240	Prallethrin	23.11	123	(100)	105	(17)	134	(9)			0.0375	0.9964
241	Penconazole	23.17	248	(100)	250	(33)	161	(50)			0.0375	0.9992
242	Mecarbam	23.46	131	(100)	296	(22)	329	(40)			0.0500	0.9986
243	Tetraconazole	23.35	336	(100)	338	(33)	171	(10)			0.0375	0.9992

Table 1. Continued.

244	Propaphos	23.92		304	(100)	220	(108)	262	(34)	0.0250	
245	Flumetralin	24.10		143	(100)	157	(25)	404	(10)	0.0250	0.9873
246	Triadimenol	24.22		112	(100)	168	(81)	130	(15)	0.0375	0.9972
247	Pretilachlor	24.67		162	(100)	238	(26)	262	(8)	0.0250	0.9980
248	Kresoxim-methyl	25.04		116	(100)	206	(25)	131	(66)	0.0125	0.9976
249	Fluazifop-butyl	25.21		282	(100)	383	(44)	254	(49)	0.0125	0.9961
250	Chlorfluazuron	25.27		321	(100)	323	(71)	356	(8)	0.0750	0.9980
251	Chlorobenzilate	25.90		251	(100)	253	(65)	152	(5)	0.0125	0.9977
252	Uniconazole	26.15		234	(100)	236	(40)	131	(15)	0.0250	0.9983
253	Flusilazole	26.19		233	(100)	206	(33)	315	(9)	0.0375	0.9983
254	Fluorodifen	26.59		190	(100)	328	(35)	162	(34)	0.0125	
255	Diniconazole	27.03		268	(100)	270	(65)	232	(13)	0.0375	0.9936
256	Piperonyl butoxide	27.46		176	(100)	177	(33)	149	(14)	0.0125	0.9919
257	Propargite	27.87		135	(100)	350	(7)	173	(16)	0.0250	0.9995
258	Mepronil	27.91		119	(100)	269	(26)	120	(9)	0.0125	0.9941
259	Dimefuron	27.82		140	(100)	105	(75)	267	(36)	0.0500	0.9885
260	Diflufenican	28.45		266	(100)	394	(25)	267	(14)	0.0125	0.9950
261	Fenazaquin	28.97		145	(100)	160	(46)	117	(10)	0.0125	0.9950
262	Phenothrin	29.08	29.21	123	(100)	183	(74)	350	(6)	0.0125	0.9950
263	Fludioxonil	28.93		248	(100)	127	(24)	154	(21)	0.0125	0.9963
264	Fenoxycarb	29.57		255	(100)	186	(82)	116	(93)	0.0750	0.9902
265	Sethoxydim	29.63		178	(100)	281	(51)	219	(36)	0.1125	0.9868
266	Amitraz	29.92		293	(100)	162	(113)	132	(104)	0.0125	
267	Anilofos	30.68		226	(100)	184	(52)	334	(10)	0.0500	0.9954
268	Acrinathrin	31.07		181	(100)	289	(31)	247	(12)	0.0250	0.9939
269	Lambda-cyhalothrin	31.11		181	(100)	197	(100)	141	(20)	0.0125	0.9982
270	Mefenacet	31.29		192	(100)	120	(35)	136	(29)	0.0375	0.9928
271	Permethrin	31.57		183	(100)	184	(14)	255	(1)	0.0250	0.9967
272	Pyridaben	31.86		147	(100)	117	(11)	364	(7)	0.0125	0.9958
273	Fluoroglycofen-ethyl	32.01		447	(100)	428	(20)	449	(35)	0.1500	0.9826
274	Bitertanol	32.25		170	(100)	112	(8)	141	(6)	0.0375	0.9931
275	Etofenprox	32.75		163	(100)	376	(4)	183	(6)	0.0125	0.9961
276	Cycloxydim	33.05		178	(100)	279	(7)	251	(4)	0.1500	0.9907
277	Alpha-cypermethrin	33.35		163	(100)	181	(84)	165	(63)	0.0250	0.9974
278	Flucythrinate	33.58	33.85	199	(100)	157	(90)	451	(22)	0.0250	0.9903
279	Esfenvalerate	34.65		419	(100)	225	(158)	181	(189)	0.0500	0.9983

Table 1. Continued.

280	Difenconazole	35.40	323	(100)	325	(66)	265	(83)	0.0750	0.9860
281	Flumioxazin	35.50	354	(100)	287	(24)	259	(15)	0.0250	
282	Flumiclorac-pentyl	36.34	423	(100)	308	(51)	318	(29)	0.0250	0.9903
SIM-D										
283	Dimefox	5.62	110	(100)	154	(75)	153	(17)	0.0375	0.9992
284	Disulfoton-sulfoxide	8.41	212	(100)	153	(61)	184	(20)	0.0250	0.9993
285	Pentachlorobenzene	11.11	250	(100)	252	(64)	215	(24)	0.0125	0.9998
286	Tri-iso-butyl phosphate	11.65	155	(100)	139	(67)	211	(24)	0.0125	
287	Crimidine	13.13	142	(100)	156	(90)	171	(84)	0.0125	0.9987
288	BDMC-1	13.25	200	(100)	202	(104)	201	(13)	0.0500	0.9978
289	Chlorfenprop-methyl	13.57	165	(100)	196	(87)	197	(49)	0.0125	0.9989
290	Thionazin	14.04	143	(100)	192	(39)	220	(14)	0.0125	0.9969
291	2,3,5,6-Tetrachloroaniline	14.22	231	(100)	229	(76)	158	(25)	0.0125	0.9994
292	Tri-n-butyl phosphate	14.33	155	(100)	211	(61)	167	(8)	0.0250	0.9972
293	2,3,4,5-Tetrachloroanisole	14.66	246	(100)	203	(70)	231	(51)	0.0125	0.9997
294	Pentachloroanisole	15.19	280	(100)	265	(100)	237	(85)	0.0125	0.9996
295	Tebutam	15.30	190	(100)	106	(38)	142	(24)	0.0250	0.9990
296	Dioxabenzofos	16.14	216	(100)	201	(26)	171	(5)	0.1250	0.9980
297	Methabenzthiazuron	16.34	164	(100)	136	(81)	108	(27)	0.1250	0.9930
298	Simetone	16.69	197	(100)	196	(40)	182	(38)	0.0250	0.9984
299	Atratone	16.70	196	(100)	211	(68)	197	(105)	0.0125	0.9988
300	Desisopropyl-atrazine	16.69	173	(100)	158	(84)	145	(73)	0.1000	0.9975
301	Terbufos sulfone	16.79	231	(100)	288	(11)	186	(15)	0.0125	0.9980
302	Tefluthrin	17.24	177	(100)	197	(26)	161	(5)	0.0125	0.9980
303	Bromocylen	17.43	359	(100)	357	(99)	394	(14)	0.0125	0.9990
304	Trietazine	17.53	200	(100)	229	(51)	214	(45)	0.0125	0.9987
305	Etrimfos oxon	17.83	292	(100)	277	(35)	263	(12)	0.0125	0.9990
306	Cycluron	17.95	89	(100)	198	(36)	114	(9)	0.0375	0.9982
307	2,6-Dichlorobenzamide	17.93	173	(100)	189	(36)	175	(62	0.0250	0.9983
308	DE-PCB 28	18.15	256	(100)	186	(53)	258	(97)	0.0125	0.9997
309	DE-PCB 31	18.19	256	(100)	186	(53)	258	(97)	0.0125	0.9997
310	Desethyl-sebuthylazine	18.32	172	(100)	174	(32)	186	(11)	0.0250	0.9985
311	2,3,4,5-Tetrachloroaniline	18.55	231	(100)	229	(76)	233	(48)	0.0250	0.9989
312	Musk ambrette	18.62	253	(100)	268	(35)	223	(18)	0.0125	
313	Musk xylene	18.66	282	(100)	297	(10)	128	(20)	0.0125	

Table 1. Continued.

314	Pentachloroaniline	18.91	265	(100)	263	(63)	230	(8)	0.0125	0.9990
315	Aziprotryne	19.11	199	(100)	184	(83)	157	(31)	0.1000	0.9979
316	Sebutylazine	19.26	200	(100)	214	(14)	229	(13)	0.0125	0.9989
317	Isocarbamid	19.24	142	(100)	185	(2)	143	(6)	0.0625	0.9965
318	DE-PCB 52	19.48	292	(100)	220	(88)	255	(32)	0.0125	0.9997
319	Musk moskene	19.46	263	(100)	278	(12)	264	(15)	0.0125	
320	Prosulfocarb	19.51	251	(100)	252	(14)	162	(10)	0.0125	0.9988
321	Dimethenamid	19.55	154	(100)	230	(43)	203	(21)	0.0125	0.9990
322	Fenchlorphos oxon	19.72	285	(100)	287	(70)	270	(7)	0.0250	0.9991
323	BDMC-2	19.74	200	(100)	202	(101)	201	(12)	0.0250	0.9912
324	Paraoxon-methyl	19.83	230	(100)	247	(93)	200	(40)	0.1000	0.9852
325	Monalide	20.02	197	(100)	199	(31)	239	(45)	0.0250	0.9988
326	Musk tibeten	20.40	251	(100)	266	(25)	252	(14)	0.0125	
327	Isobenzan	20.55	311	(100)	375	(31)	412	(7)	0.0125	0.9995
328	Octachlorostyrene	20.60	380	(100)	343	(94)	308	(120)	0.0125	0.9997
329	Pyrimitate	20.59	305	(100)	153	(116)	180	(49)	0.0125	
330	Isodrin	21.01	193	(100)	263	(46)	195	(83)	0.0125	0.9982
331	Isomethiozin	21.06	225	(100)	198	(86)	184	(13)	0.0250	0.9970
332	Trichloronat	21.10	297	(100)	269	(86)	196	(16)	0.0125	0.9989
333	Dacthal	21.25	301	(100)	332	(31)	221	(16)	0.0125	0.9995
334	4,4-Dichlorobenzophenone	21.29	250	(100)	252	(62)	215	(26)	0.0125	0.9959
335	Nitrothal-isopropyl	21.69	236	(100)	254	(54)	212	(74)	0.0250	0.9911
336	Musk ketone	21.70	279	(100)	294	(28)	128	(16)	0.0125	
337	Rabenzazole	21.73	212	(100)	170	(26)	195	(19)	0.0125	0.9931
338	Cyprodinil	21.94	224	(100)	225	(62)	210	(9)	0.0125	0.9977
339	Dicapthon	22.44	262	(100)	263	(10)	216	(10)	0.0625	0.9946
340	DE-PCB 101	22.62	326	(100)	254	(66)	291	(18)	0.0125	0.9994
341	MCPA-butoxyethyl ester	22.61	300	(100)	200	(71)	182	(41)	0.0125	0.9966
342	Isocarbophos	22.87	136	(100)	230	(26)	289	(22)	0.0250	0.9995
343	Phorate sulfone	23.15	199	(100)	171	(30)	215	(11)	0.0125	0.9946
344	Chlorfenethol	23.29	251	(100)	253	(66)	266	(12)	0.0125	0.9975
345	Trans-nonachlor	23.62	409	(100)	407	(89)	411	(63)	0.0125	0.9990
346	Dinobuton	23.88	211	(100)	240	(15)	223	(15)	0.2500	0.9788
347	DEF	24.08	202	(100)	226	(51)	258	(55)	0.0250	0.9971
348	Flurochloridone	24.31	311	(100)	187	(74)	313	(66)	0.0250	0.9979
349	Bromfenvinfos	24.62	267	(100)	323	(56)	295	(18)	0.0125	0.9937

Table 1. Continued.

350	Perthane	24.81	223	(100)	224	(20)	178	(9)	0.0125	0.9974
351	DE-PCB 118	25.08	326	(100)	254	(38)	184	(16)	0.0125	0.9985
352	4,4-Dibromobenzophenone	25.30	340	(100)	259	(30)	184	(179)	0.0250	0.9945
353	Flutriafol	25.31	219	(100)	164	(96)	201	(7)	0.0250	0.9985
354	Mephosfolan	25.29	196	(100)	227	(49)	168	(60)	0.0250	0.9890
355	DE-PCB 153	25.64	360	(100)	290	(62)	218	(24)	0.0 125	0.9992
356	Diclobutrazole	25.95	270	(100)	272	(68)	159	(42)	0.0500	0.9957
357	Disulfoton sulfone	26.16	213	(100)	229	(4)	185	(11)	0.0250	0.9966
358	Hexythiazox	26.48	227	(100)	156	(158)	184	(93)	0.1000	0.9981
359	DE-PCB 138	26.84	360	(100)	290	(68)	218	(26)	0.0 125	0.9991
360	Resmethrin-1	27.26	171	(100)	143	(83)	338	(7)	0.0250	0.9823
361	Cyproconazole	27.23	222	(100)	224	(35)	223	(11)	0.0 125	0.9983
362	Resmethrin-2	27.43	171	(100)	143	(80)	338	(7)	0.0250	0.9953
363	Phthalic acid, benzyl butyl ester	27.56	206	(100)	312	(4)	230	(1)	0.0 125	0.9973
364	Clodinafop-propargyl	27.74	349	(100)	238	(96)	266	(83)	0.0250	0.9820
365	Fenthion sulfoxide	28.06	278	(100)	279	(290)	294	(145)	0.0500	0.9863
366	Fluotrimazole	28.39	311	(100)	379	((60)	233	(36)	0.0 125	0.9981
367	Fluroxypr-1-methylheptyl ester	28.45	366	(100)	254	(67)	237	(60)	0.0250	0.9974
368	Fenthion sulfone	28.55	310	(100)	136	(25)	231	(10)	0.0500	0.9839
369	Triphenyl phosphate	28.65	326	(100)	233	(16)	215	(20)	0.0 125	0.9990
370	Metamitron	28.63	202	(100)	174	(52)	186	(12)	0.5000	0.9994
371	DE-PCB 180	29.05	394	(100)	324	(70)	359	(20)	0.0 125	0.9989
372	Tebufenpyrad	29.06	318	(100)	333	(78)	276	(44)	0.0 125	0.9965
373	Cloquintocet-mexyl	29.32	192	(100)	194	(32)	220	(4)	0.0 125	0.9915
374	Lenacil	29.70	153	(100)	136	(6)	234	(2)	0.0125	0.9967
375	Bromuconazole-1	29.90	173	(100)	175	(65)	214	(15)	0.0250	0.9916
376	Desbrom-leptophos	30.15	377	(100)	171	(97)	375	(72)	0.0 125	0.9990
377	Bromuconazole-2	30.72	173	(100)	175	(67)	214	(14)	0.0250	0.9994
378	Nitralin	30.92	316	(100)	274	(58)	300	(15)	0.1250	0.9907
379	Fenamiphos sulfoxide	31.03	304	(100)	319	(29)	196	(22)	0.0500	0.9561
380	Fenamiphos sulfone	31.34	320	(100)	292	(57)	335	(7)	0.0500	0.9954
381	Fenpiclonil	32.37	236	(100)	238	(66)	174	(36)	0.0500	0.9981
382	Fluquinconazole	32.62	340	(100)	342	(37)	341	(20)	0.0 125	0.9991
383	Fenbuconazole	34.02	129	(100)	198	(51)	125	(31)	0.0250	0.9896

*Numbers in parentheses are the relative abundance.

Calibration standard: Calibration standard was prepared at different concentrations (0.01, 0.05, 0.5, 1 and 2 ppm) by a dilution of the stock standard solution with hexane, corresponding to the expected range of concentrations found in the samples. It was then used to calibrate (retention time and area count) the instrument response with respect to analyte concentration.

Apparatus

GC-MS system

A QP 2010 gas chromatograph was connected to a mass selective detector and equipped with an auto sampler (Shimadzu, Japan). The column used was a capillary column (DB-1701, 30 m x 0.25 mm x 0.25 mm). The GC-MS operating condition comprised column temperature (which increased from 40°C held for 1 min at 30°C min^{-1} to 130°C at 5°C min^{-1}, 250°C at 10°C min^{-1} and was further increased to 300°C, which was held for 5 min); carrier gas (helium and purity 99.999%, flow rate 1.2 ml min^{-1}); injection port temperature (290°C); injection volume (1 ml); injection mode (split less, purge after 1.5 min ionization voltage, and 70 eV); ion source temperature (230°C); GC-MS interface temperature (280°C) and selected ion monitor mode (for each compound, one quantifying ion and two to three qualifying ions were selected).

Peak identification/library search

Individual peak was identified in different internal libraries in GC-MS. It had the following libraries:

(1) PESTI NCI Lib. (2) PESTI EI Lib

Confirmation and quantification

Spiking

Identifications of the analyte were confirmed by spiking with a known standard.

Laboratory reagent blank

An aliquot of reagent grade water was treated exactly as a sample including exposure to all glassware, equipment, solvents and reagents used with the sample matrix. As a result, no analyte peak was detected.

Laboratory fortified sample matrix

An aliquot of a sample matrix (blood) was prepared to which known quantities of the pesticides were added in the laboratory in ppm range. This laboratory fortified matrix was analyzed exactly like the sample. As a result, 15% of the samples (minimum) were fortified with a known concentration of pesticide and percent recovery was calculated. Extraction and clean up was done as mentioned and the recovery of the pesticides over the background values obtained from unfortified samples were more than 80%. Standard deviation and coefficient of variation were less than 10 indicating repeatability of the method. All calculations were done as described in US EPA (1985) method and the amount of residues in samples was obtained in mg/l (ppm) of the whole blood.

RESULTS AND DISCUSSION

A total of 27 blood samples were analyzed for detection of pesticides using multi pesticide resi-due method to determine the presence or absence of pesticide in occupationally exposed workers. Two pesticides (chlorpyrifos and pyributicarb) were detected in two of 27 blood samples.

Sample analysis results of sample no: 1 to 27

No pesticide residues were traced in sample no: 1 to 13, 15 to 18 and 20 to 27. Sample no: 14 and 19 were positive for chlorpyrifos and pyributicarb as shown in Figure 1 and Table 2 and Figure 2 and Table 3, respectively.

Chlorpyrifos

It was detected in one of the 27 blood samples which constitute 4% of the total samples analyzed with a concentration of 0.009 mg/l of the whole blood.

Pyributicarb

This was detected in one of the 27 blood samples, that is, (4)% of the total samples analyzed with a concentration of 0.001 mg/l of the whole blood. Due to the fact that the frequency and concentration of residues detected in blood samples were very low, the results do not agree to those reported by Mathur et al. (2005) in 20 blood samples collected from 4 villages in Punjab, India, which were analyzed for both 14 organochlorines and organophosphorus pesticides.

Chlorpyrifos (85%) of the samples analysed, indicated regular and widespread exposure to this pesticide. Concentration of chlorpyrifos was very low as compared to that detected by Schafer et al. (2004), who reported that chlorpyrifos and methyl parathion were found at levels up to 4.5 times higher than what the U.S government deems acceptable. The reason for the very low concentration of these pesticides can be hypothe-sized in the off season of pesticide application, because the area selected for the study is covered by wheat (*Triticum aestivum*) from November to May and no spray is recommended on wheat crop.

Method authenticity

The study covers a detection of 383 (Table 1) pesticides simultaneously (Pang et al., 2006). Liquid–liquid extraction method was adopted in this study for the detection of several classes of pesticides and their primary metabolites, which are simple, rapid and sensitive as a means of exposure analysis in a human population. Most recent exposure analysis studies have focused on blood serum

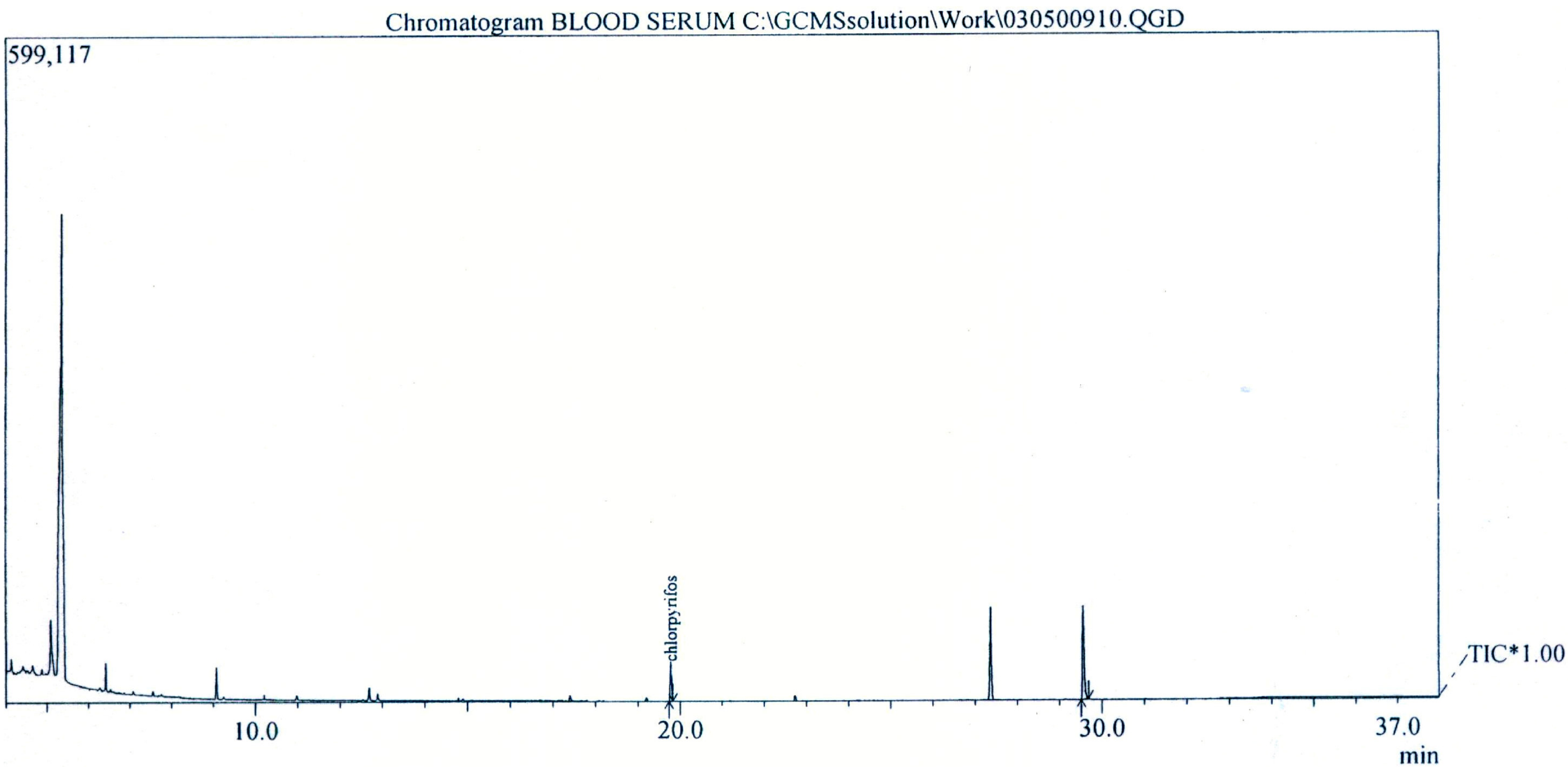

Figure 1. Analysis of pesticide residues in blood sample no.14.

or plasma as the preferred matrices of interest (Arrebola et al., 2001; Covaci and Schepens, 2001; Guardino et al., 1996; Liu et al., 1999; Waliszewski and Szymczynski, 1991). How-ever, these studies have not acknowledged the fact that representative concentrations of highly lipophilic pesticides are more likely to be found in the whole blood. Additionally, since blood is a complex matrix, quantification methods of pesti-cide exposure have been primarily focused on a single group of pesticides (that is, organophosphates or pyrethroids).

Waliszewski and Szymczynski developed a technique for analyzing organochlorines in 5 ml of the whole blood using GC with an electron capture detector (Waliszewski and Szymczynski, 1991). Their reported recoveries were compa-rable. However, they did not extrapolate their method for the whole blood to a human population for exposure analysis. More recently, Ramesh and Ravi developed a method of analysis for the

Table 2. Pesticide detected in blood sample no. 14.

Peak report TIC				
Peak#	**R. time**	**Area**	**Height**	**Name**
1	19.784	74662	35249	Chlorpyrifos
		74662	35249	

Detected = Chlorpyrifos.

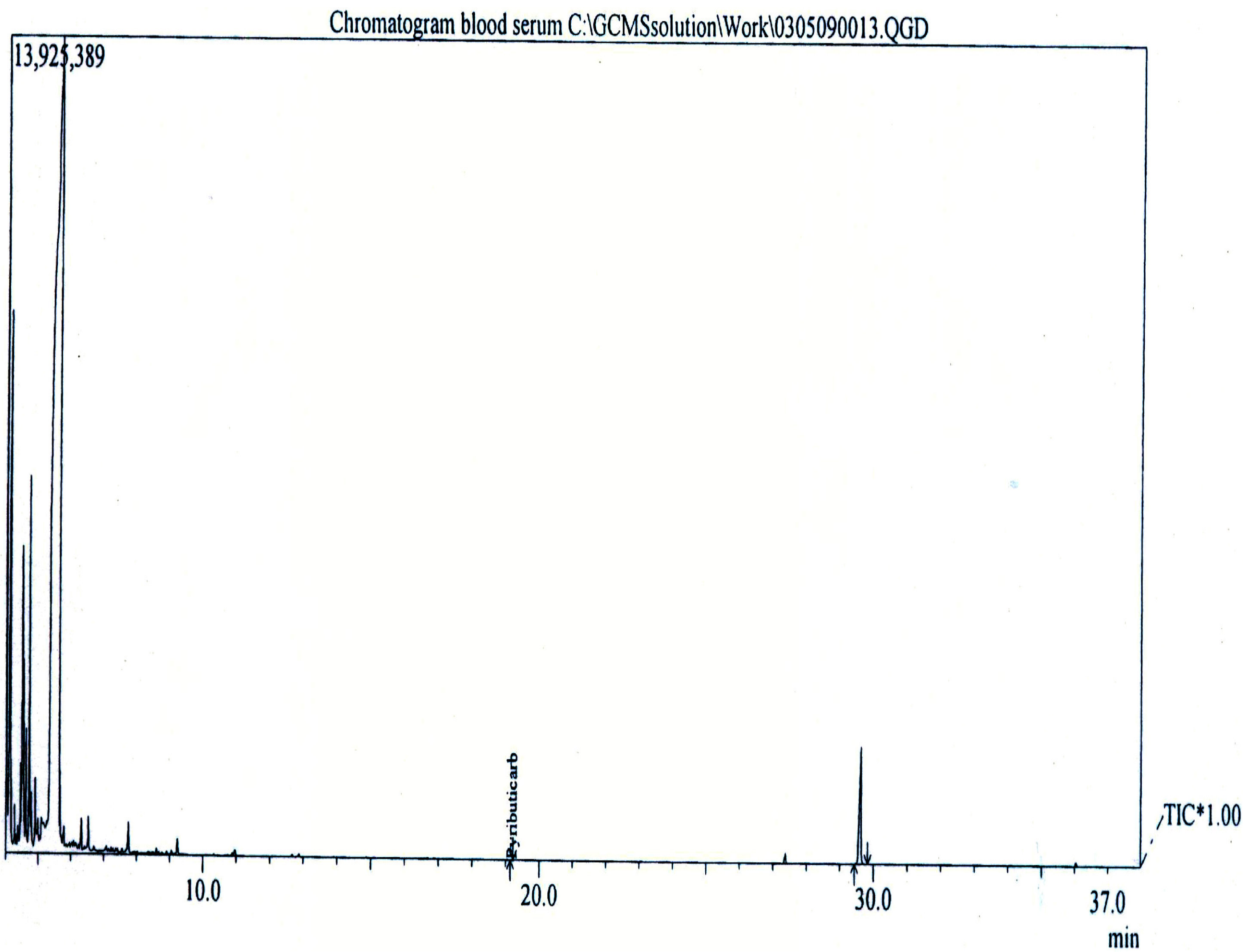

Figure 2. Analysis of pesticide residues in blood sample no.19.

Table 3. Pesticide detected in blood sample no. 19.

Peak report TIC				
Peak#	**R. time**	**Area**	**Height**	**Name**
1	19.187	14265	5105	Pyributicarb
		14265	5105	

*Detected = Pyributicarb.

whole blood using GC–MS, exclusively investigating pyrethroid parent compounds with similar chemical structures (Ramesh and Ravi, 2004). Again, the study's multi-class method is comparable for the corresponding compounds, and it also included several common metabolites that were not incorporated in the Ramesh and Ravi's method. Furthermore, when Ramesh and Ravi applied their method of analysis to a human population that is continuously exposed to different pyrethroid formulations, none of the 45 blood samples analyzed contained detectable levels of pyrethroids. The authors attributed their findings to rapid excretion of the compounds or limitations of the experimental design. Also, they have not found the pyrethroid compounds in any of their samples. The reason may be the off time sampling, that is, March which is a non spraying period comprised most of the areas that are covered by wheat and no spray of any chemical is recommended on wheat crop. Moreover, a very low quantity of only two pesticides in two samples, suggest the degradation of pesticides in the body of a spray worker over the period of pesticide application season.

Conclusion

Clearly, biological monitoring using blood as a biomarker of pesticide exposure has merited attention from the scientific community, and its significance is reflected in the wealth of studies concerned with the detection and quantification of low-level exposures. However, the ubiquitous nature of pesticides requires analytical methods that sensitively and efficiently detect compounds from several diverse classes simultaneously to more accurately reflect a person's exposure. Though incidental exposures generally result in very low concentrations as a consequence of rapid excretion, the presence of these compounds signifies health risks for adults and children. Finally, the study concludes that the method for the whole blood analysis is a suitable tool in investigating the exposure of spray men to environmental pollutants.

ACKNOWLEDGEMENT

The authors are thankful to the Higher Education Commission of Pakistan, for sponsoring this research work.

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Annexure 1. Names and address of individuals randomly selected at District Vehari, Punjab (Pakistan).

S/No.	Sample code	Date	Name	Father's name	Sex	Spray experience (Years)	Age (Years)	Address	PH#
1	Blood 1	15/03/09	Muhammad Ilyas	Muhammad Iqbal	Male	4	25	166/WB; District Vehari.	+923087529430
2	Blood 2	15/03/09	Muhammad.Arshad	G. Muhammad	Male	5	27	166/WB, District Vehari	+923013763465
3	Blood 3	15/03/09	Zahid Ali	Ghulam Rasool	Male	3	18	140/10.R; Distt. Vehari	+923347824423
4	Blood 4	15/03/09	Allah Wasaya	Allah Ditha	Male	1	35	166/WB; District Vehari	NA
5	Blood 5	15/03/09	Muhammad Azam	Liaqat	Male	7	16	166/WB; District Vehari	+923023735936
6	Blood 6	15/03/09	Wazir Ahmed	Falak Shair	Male	0	17	166/WB; District Vehari	NA
7	Blood 7	16/03/09	Muhammad Ajmal	Sikandar	Male	5	25	166/WB; District Vehari	NA
8	Blood 8	16/03/09	Muhammad Safdar	Siafal	Male	9	25	116/10.R; Distt. Vehari	NA
9	Blood 9	16/03/09	Nazar Hussain	Amir Bux	Male	6	40	166/WB; District Vehari	NA
10	Blood 10	16/03/09	Muhammad Ramzan	Karam Hussain	Male	0	26	Mondhi Khoh, Vehari	NA
11	Blood 11	16/03/09	Falak Shair	Allah Ditha	Male	5	50	116/10.R; District Vehari	NA
12	Blood 12	16/03/09	Muhammad Akram	Muhammad Sadiq	Male	4	25	166/WB; District Vehari	+923037834091
13	Blood 13	16/03/09	Abdul Razaq	Muhammad Anwar	Male	2	35	170/WB; District Vehari	+923008736035
14	Blood 14	16/03/09	Ibrahim	Gulam Farid	Male	8	35	Mondhi Khoh; Vehari	NA
15	Blood 15	16/03/09	Khadim Hussain	Allah Bux	Male	4	28	170/WB; District Vehari	NA
16	Blood 16	16/03/09	Allah Bux	Pir Bux	Male	6	50	170/WB; District Vehari	NA
17	Blood 17	16/03/09	Muhammad Rizwan	Muhammad Tufail	Male	3	18	170/WB; District Vehari	NA
18	Blood 18	16/03/09	Muhammad Ijaz	Allah Ditha	Male	3	18	170/WB; District Vehari	NA
19	Blood 19	16/03/09	Muhammad Ismaiel	Gulam Hussain	Male	3	25	170/WB; District Vehari	+923027830217
20	Blood 20	16/03/09	Muhammad Munir	Allah Ditha	Male	5	24	170/WB; District Vehari	NA
21	Blood 21	16/03/09	Irfan.	Shamir	Male	4	20	166/WB; District Vehari	NA
22	Blood 22	16/03/09	Muhammad Bakir	Muhammad Aslam	Male	2	18	166/WB; District Vehari	NA
23	Blood 23	17/03/09	Khizar Hayat	Muhammad Saifal	Male	3	18	166/WB; District Vehari	NA
24	Blood 24	16/03/09	Muhammad Safdar	Muhammad Gazi	Male	4	20	166/WB; District Vehari	NA
25	Blood 25	16/03/09	Muhammad Riaz	Guiam Rasul	Male	6	26	166/WB; District Vehari	NA
26	Blood 26	16/03/09	Muhammad Arif	M. Salabat	Male	4	25	166/WB; District Vehari	NA
27	Blood 27	16/03/09	Ali Gull	Amir Bux	Male	0	29	166/WB; District Vehari	+923073143017

NA= not available.

Annexure 2. Total pesticide detected in total samples.

S/No.	Sample code	Name of pesticide	Positive sample	Total samples	Positive samples (%)	Peak area	Retention time	Concentration (mg/l)
1	Blood 14	Chlorpyrifos	1	24	4	74662	19.784	0.009
2	Blood 19	Pyributicarb	1	24	4	14265	19.187	0.001