

Chemicals

All standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and certified to have a purity level exceeding 98%. Methanol (MeOH) and LC-grade ultrapure water were procured from Merck (99.8–100%, Darmstadt, Germany). Oasis HLB cartridges (3 cc, 60 mg) for the process of solid phase extraction (S.P.E.) were acquired from Waters Corporation (Milford, MA, U.S.A.).

Sample Preparation

Each participant's blood sample was collected in a 3 mL EDTA tube (K2EDTA 5.4 mg, B.D. Vacutainer, Plymouth, U.K.). A 1 mL blood sample was transferred to a glass tube, and 3 mL of a 0.1 M phosphate buffer solution with a pH of 7.4 and an internal standard (10 ng/mL dimethoate-d₆) were added. The blood sample was centrifuged (3000 rpm, 10 minutes), and the supernatant was collected. Oasis HLB cartridges (3 cc, 60 mg) were conditioned with 2 mL of methanol followed by 2 mL of distilled water. After adding the sample, the cartridges were washed with 5% methanol, and eluents were collected using 2 mL of methanol. The eluents were evaporated under a gentle nitrogen flow until dry and reconstituted with 150 μ L of methanol. The extracts were analyzed using an optimized LC/MS/MS method.

LC-MS/MS analysis

A total of 144 pesticides in blood samples were analyzed using a Shimadzu 8040 LC-MS/MS Systems instrument (Kyoto, Japan).

The analytes were chromatographically separated at a 0.4 mL/min flow rate on the Shim-pack column (150x2.0 mm I.D.) purchased from Shimadzu (Tokyo, Japan). All pesticides were adequately separated using the following gradient program: The mobile phase consisted of 5 mM ammonium formate in water (A) and 5 mM ammonium formate in methanol (B). The gradient was started for the positive mode with 10% B and held at a flow rate of 0.5 mL/min for 10 min; then, the flow rate was increased to 1 mL/min with 90% B and held at these conditions for 5 min, after 15 min the flow rate was returned to 0.5 mL/min with 10% B. The eluent was diverted to waste for the first 1.5 minutes. The total run time per sample was 18 minutes. All selected analyses were performed in positive ionization

mode (ESI+). The analyzed compounds were identified and quantified using two characteristic transitions (M.R.M. mode). The retention time, precursor ions, and product ions of the 144 pesticides used are listed in the Supplementary file (Table S1).

Calibration was prepared by spiking with the following concentrations: 0.05, 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 80, and 100 ng/mL in blank blood samples. The deuterated internal standard was added to the blood (10 ng/mL, dimethoate-d6). The limit of detection (L.O.D.) and the limit of determination (L.O.Q.) values were determined based on the signal-to-noise ratios of 3 and 10, respectively. The L.O.D. values for all O.P.s were in the range of 0.01–0.02 ng/mL of blood, and the L.O.Q. values for all O.P.s were in the range of 0.05-0.18 ng/mL.

Table S1. Analysis parameters for LC-MS/MS instruments of organophosphorus pesticides (OPs)

| OPs | Class of Pesticides | Retention time | Precursor Ion | Fragment ion-1 | Fragment ion-2 |
|----------------------|------------------------------|----------------|---------------|----------------|----------------|
| Fenoxaprop-P-ethyl | Herbicide | 13.491 | 361.800 | 288.100 | 91.250 |
| Triclorfon | Insecticide | 7.186 | 256.850 | 109.000 | 220.700 |
| Cymoxanil | Fungicide | 8.442 | 199.100 | 128.150 | 111.100 |
| Chlormequat Chloride | Herbicide | 0.909 | 122.000 | 58.200 | 63.100 |
| Clothianidine | Insecticide(neonicotinoid) | 7.338 | 249.700 | 132.100 | 169.100 |
| Dimethoate | Insecticide | 7.849 | 229.600 | 125.150 | 199.050 |
| Acetamiprid | neonicotinoid insecticide | 7.992 | 222.600 | 56.200 | 126.100 |
| Chloridazon | herbicide | 7.990 | 221.600 | 104.100 | 92.200 |
| Dichlorfos | Insecticide(organophosphate) | 10.300 | 220.900 | 109.150 | 127.150 |
| Carbaryl | Insecticide(carbamate) | 10.776 | 202.050 | 145.200 | 127.150 |
| Ethiofencarb | Insecticide(carbamate) | 10.957 | 225.800 | 107.150 | 164.200 |
| Carboxin | Anilide fungicide | 10.754 | 235.600 | 143.100 | 87.100 |
| Flutriafol | Fungicide | 10.643 | 302.100 | 123.200 | 109.200 |
| Atrazine | Herbicide(triazine) | 10.647 | 215.650 | 174.150 | 43.150 |
| Diuron | Herbicide(phenylureas) | 10.872 | 232.800 | 72.200 | 160.100 |
| Diphenamid | Herbicide | 10.936 | 239.700 | 134.200 | 167.200 |
| Diethofencarb | Fungicide(carbamate) | 11.896 | 267.800 | 226.200 | 180.200 |
| Azinphos-methyl | Insecticide(organophosphate) | 11.463 | 317.700 | 160.150 | 132.150 |
| Azoxystrobin | Fungicide | 11.696 | 403.800 | 372.250 | 329.100 |
| Fenamidone | Foliar fungicide | 11.994 | 311.800 | 92.200 | 236.200 |
| Boscalid | Anilide fungicide | 11.568 | 343.100 | 307.200 | 140.000 |
| Dimethomorph | Fungicide | 11.679 | 387.700 | 301.150 | 165.200 |
| Dimethenamid | Herbicide | 11.526 | 275.600 | 244.200 | 168.200 |
| Cyproconazole | Fungicide(triazole) | 12.035 | 292.100 | 70.200 | 125.150 |
| Bromuconazole | Fungicide(triazole) | 12.399 | 378.000 | 159.000 | 161.000 |

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|---------------------------|---------------------------------|--------|---------|---------|---------|
| Fenbuconazole | Fungicide(triazole) | 12.633 | 337.150 | 70.250 | 125.100 |
| Fenarimol | Fungicide(pyrimidine) | 12.499 | 330.800 | 268.100 | 139.100 |
| Dichlofluanid | Fungicide(sulfamide) | 12.495 | 350.000 | 224.050 | 123.200 |
| Epoxiconazole | Fungicide(azole) | 12.598 | 329.800 | 101.200 | 121.200 |
| Alachlor | Herbicide(chloroacetanilide) | 12.737 | 269.700 | 238.250 | 162.250 |
| Fenamiphos | Organophosphate insecticide | 12.338 | 303.600 | 217.100 | 202.100 |
| Acetochlor | herbicide | 12.664 | 269.900 | 224.200 | 148.200 |
| Buprimate | Pyrimidine fungicide | 12.783 | 316.700 | 166.200 | 108.100 |
| EPTC | Herbicide | 12.982 | 190.100 | 86.000 | 128.200 |
| Fenthion | Insecticide(thiophosphate) | 13.108 | 279.000 | 247.100 | 169.000 |
| Diniconazole | Fungicide | 13.349 | 326.100 | 70.200 | 159.100 |
| Diazinon | Insecticide(pyrimidine) | 13.093 | 304.600 | 169.100 | 97.100 |
| Difenacozole | Fungicide(triazole) | 13.326 | 405.800 | 251.100 | 111.100 |
| Cyprodinil | Fungicide | 13.262 | 225.700 | 93.150 | 108.200 |
| Cycloate | Herbicide | 13.554 | 216.150 | 154.200 | 134.200 |
| Diclofop Methyl | Herbicide | 13.792 | 358.100 | 281.100 | 120.200 |
| Dioxathion | Organophosphate insecticide | 13.608 | 473.800 | 97.100 | 153.100 |
| Buprofezin | Insecticide | 13.774 | 305.700 | 57.150 | 201.200 |
| Diafenthiuron | Insecticide | 14.123 | 384.900 | 329.300 | 278.250 |
| Fenpropathrin | Insecticide(pyrethroid) | 14.063 | 350.150 | 125.200 | 57.200 |
| Chlorfluazuron | Insecticide(benzoylurea) | 14.197 | 539.800 | 382.950 | 158.150 |
| Cypermethrin | Insecticide(pyrethroid) | 14.182 | 433.300 | 191.050 | 193.150 |
| Deltamethrin | Insecticide(pyrethroid) | 14.301 | 522.800 | 281.000 | 506.200 |
| Abamectin | Insecticide | 14.462 | 890.600 | 305.350 | 145.150 |
| Fenpyroximate | Acaricide(pyrazole) | 14.286 | 421.700 | 366.300 | 135.100 |
| Carbosulfan | Insecticide(carbamate) | 14.783 | 380.700 | 118.250 | 160.250 |
| Fenazaquin | Acaricide(quinazoline) | 14.834 | 306.900 | 57.200 | 161.200 |
| Bifenthrin | Insecticide(pyrethroid) | 15.109 | 440.200 | 181.200 | 166.100 |
| Etofenprox | Insecticide(pyrethroid) | 15.206 | 393.800 | 177.200 | 107.150 |
| bensulfuron-methyl | Herbicide | 11.383 | 410.800 | 149.200 | 182.200 |
| Clodinafob-propargyl | Herbicide(pyridine) | 12.829 | 349.700 | 266.000 | 238.100 |
| Ethofumesate | Herbicide | 11.951 | 287.100 | 121.200 | 258.800 |
| Ethoprophos | organothiophosphate insecticide | 12.615 | 242.800 | 131.000 | 172.900 |
| chlorpyrifos-ethyl | organothiophosphate insecticide | 14.091 | 349.700 | 197.900 | 97.000 |
| chlorpyrifos-methyl | organothiophosphate insecticide | 13.532 | 321.900 | 125.000 | 289.900 |
| Chlorsulfuron | Herbicide | 7.757 | 357.800 | 141.200 | 167.200 |
| Dazomet | Fumigant | 4.921 | 163.000 | 89.900 | 119.900 |
| Nicosulfuron | Herbicide(pyridine) | 6.753 | 411.100 | 182.200 | 106.200 |
| Propamocarb hydrochloride | Fungicide | 5.331 | 188.800 | 102.200 | 144.200 |
| Oxamyl | carbamate pesticide | 5.179 | 236.850 | 72.250 | 90.250 |

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|-------------------------|---------------------------------|--------|---------|---------|---------|
| Methomyl | Insecticide(carbamate) | 5.666 | 162.800 | 88.200 | 106.200 |
| Monocrotophos | organophosphate insecticide | 6.361 | 224.100 | 127.100 | 98.200 |
| Imidachloropid | Insecticide(neonicotinoid) | 7.170 | 256.100 | 209.150 | 175.150 |
| Imazethapyr | Herbicide(pyridine) | 6.329 | 289.800 | 230.200 | 177.200 |
| Foramsulfuron | Herbicide(benzamide) | 8.939 | 453.000 | 182.200 | 139.100 |
| Oxadixyl | Anilide fungicide | 9.662 | 279.150 | 219.250 | 132.250 |
| Monolinuron | Herbicide | 10.204 | 215.000 | 126.100 | 148.200 |
| Pirimicarb | Insecticide(carbamate) | 10.382 | 238.700 | 72.150 | 182.300 |
| Metalaxyl M | Anilide fungicide | 11.378 | 279.750 | 220.250 | 192.250 |
| Isoxaflutole | Herbicide(isoxazole) | 11.418 | 377.300 | 251.100 | 219.850 |
| Methidathion | organothiophosphate insecticide | 11.686 | 303.000 | 144.900 | 85.200 |
| Phosmet | organothiophosphate insecticide | 11.204 | 318.050 | 160.150 | 133.150 |
| Myclobutanil | Fungicide(triazole) | 11.817 | 288.900 | 70.200 | 288.900 |
| Malathion | organothiophosphate insecticide | 12.240 | 330.800 | 127.200 | 99.100 |
| Propyzamide | Herbicide(benzamide) | 12.325 | 256.050 | 190.050 | 173.050 |
| Pyridaphenthion | organothiophosphate insecticide | 12.321 | 340.800 | 189.150 | 92.200 |
| Flusilazole | organosilicon fungicide | 12.711 | 315.800 | 247.200 | 165.150 |
| Metolachlor | Herbicide | 12.733 | 283.800 | 252.200 | 176.250 |
| Prometryn | Herbicide | 12.642 | 241.800 | 158.100 | 200.200 |
| Penconazole | Fungicide(triazole) | 12.958 | 284.250 | 70.250 | 159.050 |
| Imazalil | Fungicide | 12.953 | 297.100 | 159.000 | 255.100 |
| Phenthoate | organothiophosphate insecticide | 12.869 | 321.100 | 135.150 | 163.250 |
| Kresoxim M | Fungicide | 12.927 | 313.900 | 222.250 | 206.200 |
| Pyraflufen-ethyl | Herbicide(pyrazole) | 12.924 | 413.100 | 339.100 | 253.100 |
| Metconazole | Fungicide(triazole) | 13.140 | 320.200 | 70.200 | 125.150 |
| Hexaconazole | Fungicide(triazole) | 13.149 | 314.100 | 70.200 | 159.100 |
| Propiconazole | Fungicide(triazole) | 12.789 | 342.100 | 159.000 | 69.200 |
| Mefenpyr-diethyl | Herbicide | 13.104 | 389.900 | 327.150 | 160.000 |
| Prochloraz | Fungicide(imidazole) | 13.204 | 375.800 | 308.100 | 266.000 |
| Phosalone | organothiophosphate insecticide | 13.195 | 368.050 | 182.150 | 111.100 |
| Pyraclostrobin | Fungicide(carbamate) | 13.120 | 387.800 | 163.100 | 104.200 |
| Pirimiphos-methyl | organothiophosphate insecticide | 13.092 | 305.800 | 108.100 | 164.200 |
| Pyrazophos | Fungicide | 13.286 | 373.800 | 222.200 | 194.200 |
| Haloxfop-2-ethoxy ethyl | Herbicide(pyridine) | 13.558 | 433.900 | 316.150 | 91.200 |
| Fluazifop- P-Butyl | Herbicide | 13.565 | 383.900 | 282.200 | 328.200 |
| Profenofos | organophosphate insecticide | 13.529 | 372.950 | 302.900 | 345.100 |
| Propaquizafop | Herbicide | 13.657 | 444.200 | 371.000 | 100.200 |
| Hexythiazox | Acaricide | 13.992 | 353.200 | 228.100 | 168.100 |
| Propargite | Acaricide | 14.064 | 368.250 | 175.200 | 231.300 |

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|------------------------|---------------------------------|--------|---------|---------|---------|
| Pyriproxyfen | Insecticide(pyridine) | 13.996 | 321.700 | 96.200 | 185.150 |
| Pendimethalin | Herbicide(aniline) | 14.179 | 282.200 | 194.100 | 212.200 |
| Pyridaben | organochlorine insecticide | 14.448 | 365.000 | 147.300 | 309.200 |
| Prothiophos | organothiophosphate insecticide | 12.936 | 344.500 | 241.100 | 133.200 |
| Pyridate | Herbicide(pyridazine) | 5.036 | 379.200 | 207.100 | 351.250 |
| Halfenprox | Insecticide(pyrethroid) | 15.459 | 494.200 | 183.200 | 155.100 |
| Fosthiazate | organothiophosphate insecticide | 10.999 | 284.100 | 104.200 | 227.700 |
| Furathiocarb | Insecticide(carbamate) | 13.652 | 382.900 | 195.200 | 252.100 |
| haloxyfop-p-methyl | Insecticide(pyridine) | 13.337 | 376.100 | 315.900 | 91.200 |
| Imazapyr | Herbicide(pyridine) | 3.710 | 261.900 | 217.200 | 78.200 |
| iodosulfuron-methyl | Herbicide | 9.480 | 508.000 | 167.200 | 141.200 |
| Imazapic | Herbicide | 8.270 | 275.900 | 231.100 | 163.200 |
| mesosulfuron-methyl | Herbicide | 9.826 | 504.200 | 182.200 | 139.200 |
| Mesotrione | Herbicide | 4.567 | 357.100 | 104.100 | 104.100 |
| Metamitron | Herbicide(triazine) | 7.753 | 203.100 | 104.200 | 174.000 |
| Metribuzin | Herbicide(triazine) | 9.480 | 215.100 | 187.100 | 84.300 |
| quizalofop-p-ethyl | Herbicide | 13.579 | 373.500 | 299.500 | 299.100 |
| Spirodiclofen | Organochlorine acaricide | 4.829 | 411.200 | 71.200 | 313.000 |
| Spiroxamine | Fungicide | 12.908 | 298.000 | 144.300 | 100.300 |
| tau fluvalinate | Insecticide(pyrethroid) | 14.358 | 520.000 | 181.200 | 208.000 |
| Tebuconazole | Fungicide(triazole) | 12.955 | 308.200 | 70.200 | 125.200 |
| Tebufenozide | Insecticide | 12.725 | 353.300 | 133.200 | 296.900 |
| Terbutryn | Herbicide(triazine) | 12.738 | 241.700 | 186.200 | 68.200 |
| Tetraconazole | Fungicide(triazole) | 12.510 | 372.100 | 159.100 | 70.200 |
| Thiacloprid | Insecticide(neonicotinoid) | 8.787 | 252.750 | 126.100 | 99.100 |
| Thiamethoxam | Insecticide(neonicotinoid) | 5.988 | 291.800 | 210.900 | 181.200 |
| thifensulfuron-methyl | Herbicide(triazine) | 7.028 | 388.100 | 167.200 | 69.100 |
| Thiometon | Organothiophosphate insecticide | 10.467 | 247.050 | 89.200 | 88.800 |
| Tolylfluanid | Fungicide(sulfamide) | 12.897 | 363.800 | 237.900 | 137.200 |
| Triadimefon | Fungicide(triazole) | 12.286 | 294.100 | 197.000 | 70.200 |
| Triasulfuron | Herbicide | 7.943 | 402.100 | 167.200 | 141.100 |
| Florasulam | Herbicide | 6.961 | 360.050 | 129.100 | 192.000 |
| Mesotrione | Herbicide | 4.547 | 357.100 | 227.900 | 104.100 |
| Thiophanate-methyl-pos | Fungicide(carbamate) | 9.453 | 343.050 | 151.100 | 118.200 |
| Metsulfuron-methyl | Herbicide(sulfonylurea) | 6.523 | 382.100 | 167.200 | 198.900 |
| Metribuzin | Herbicide(triazine) | 9.474 | 214.800 | 187.000 | 84.100 |
| Fenoxycarb | Insecticide(carbamate) | 12.801 | 302.150 | 88.200 | 116.100 |
| Triflumizole | Fungicide(imidazole) | 13.507 | 346.500 | 278.200 | 73.000 |
| Trifloxystrobin | Fungicide | 13.341 | 409.150 | 186.200 | 145.100 |
| Fipronil | Insecticide(pyrazole) | 12.654 | 434.900 | 329.900 | 249.9 |

Gas Chromatographic (GC) analysis

Gas chromatographic (GC) analysis was performed using an Agilent (UK) model 6890N gas chromatograph equipped with an ECD detector. The chromatographic separation of OCs was carried out using a 60 m X 0.32 mm D.I. Hewlett-Packard HP-5 fused silica capillary column (Agilent, U.S.A.). The operating conditions were: injector temperature 250 °C; detector 300 °C; column 70-280 °C (2 min at 70 °C; 25 °C/min, from 70 to 150 °C; 3 °C/min, from 150 to 200 °C; 80C/min, from 200 to 280 °C; 10 min at 280 °C. Helium was used as the carrier gas.

The analyzed organochlorine compounds (OC) and their retention times and calibration ranges are listed in the Supplementary file (Table S2). Excellent linearity was obtained in the concentration range studied, with correlation coefficients between 0.9970 and 0.9999. To determine the quality of the method, a recovery study was performed on over-spiked replicates of blank blood samples. The method validation studies were performed at 1-100 ng/ml for blood samples, depending on the type of pesticide, and showed recoveries ranging from 64 % to 109.7 %. Control experiments with reagents were performed prior to analysis. The LOQ values for OCs analyzed were in the range of 1-25 ng/mL and the LOD values for OCs were in the range of 0.3-8 ng/mL for GC analysis.

Table S2: Analysis parameters for Gas Chromatography device of organochlorine compounds

| Ocs | Retention time | Calibration range (ng/mL) |
|------------------|----------------|---------------------------|
| alfa-HCH | 14.520 | 5-100 ng/mL |
| HCB | 14.858 | 1-100 ng/mL |
| β-HCH | 15.745 | 10-100 ng/mL |
| δ - HCH | 15.989 | 5-100 ng/mL |
| γ - HCH | 17.164 | 5-100 ng/mL |
| PCB-28 | 18.801 | 10-100 ng/mL |
| PCB-52 | 20.536 | 5-100 ng/mL |
| <i>o,p'</i> -DDE | 24.419 | 10-100 ng/mL |
| <i>p,p'</i> -DDE | 24.566 | 1-100 ng/mL |
| PCB-101 | 24.668 | 5-50 ng/mL |
| <i>o,p'</i> -DDD | 25.766 | 5-100 ng/mL |
| <i>p,p'</i> -DDD | 26.048 | 5-100 ng/mL |
| PCB-118 | 26.896 | 5-100 ng/mL |
| PCB-153 | 27.167 | 25-100 ng/mL |
| <i>o,p'</i> -DDT | 27.235 | 5-100 ng/mL |
| <i>p,p'</i> -DDT | 28.276 | 10-100 ng/mL |
| PCB-138 | 28.398 | 5-100 ng/mL |
| PCB-202 | 29.732 | 1-100 ng/mL |
| PCB-180 | 30.221 | 5-100 ng/mL |