

# Application News

## No.L502

### Supercritical Fluid Extraction / Chromatography

## Analysis of Residual Pesticides in Agricultural Products Using Nexera UC Off-Line SFE-GC/MS System

Since enforcement of the positive list system for residual pesticides in foods in 2006 in Japan, over 800 pesticides have been included in the system. Consequently, there is now a strong demand for effective analytical methods encompassing any sample pretreatment steps that are capable of inspecting large numbers of pesticides. Conventionally, analysis of residual pesticides in foods has involved pesticide extraction by a solvent extraction method before analysis by LC/MS or GC/MS. The problem with solvent extraction methods is that sample pretreatment requires a substantial amount of time and effort, and large quantities of organic solvents are used. Supercritical fluid extraction (SFE) that uses supercritical carbon dioxide as the extraction solvent provides good extraction efficiency, where the solvent is similar to gas in terms of low viscosity and high diffusivity, and similar to fluid in terms of high solubility. This allows for extraction within a short period of time. This extraction method is also less damaging to the environment since it uses a smaller amount of organic solvent compared to conventional solvent extraction methods. We introduce an example GC/MS analysis of pesticides extracted from an agricultural products using the Nexera UC off-line SFE system.

### Off-Line SFE System

Fig. 1 shows the principle behind operation of the Nexera UC off-line SFE system. An extraction vessel filled with a sample is placed in the SFE unit, and is heated to 40 °C (Fig. 1 A). The extraction vessel is then filled with supercritical carbon dioxide, and the target components are extracted statically without pumping the liquid (Fig. 1 B). After static extraction, dynamic extraction is performed by pumping supercritical carbon dioxide through the extraction vessel (Fig. 1 C). After trapping the extract material in the trap column, eluate that contains the target components is then collected in the fraction collector (Fig. 1 D).

### Sample Preparation

The QuEChERS method that prioritizes simplicity and speed is widely used to pretreat agricultural products for residual pesticide analysis. While there is a special kit available for the QuEChERS method, sample preparation for this kit requires a large number of process steps including reagent addition, solvent extraction, purification by dispersive solid phase extraction, and centrifugal separation.

Meanwhile, as shown in Fig. 2, sample preparation for the Nexera UC off-line SFE system only involves mixing 1 g of agricultural product pulverized in a mixer with 1 g of dehydrating agent\*, then filling the extraction vessel with this mixture. This not only results in improved productivity and a reduced environmental burden, but also avoids human errors involved in the sample pretreatment process. Using a specially designed rack changer also allows for extraction of a maximum of 48 samples consecutively.

\* "Miyazaki Hydro-Protect" Patent No. 3645552

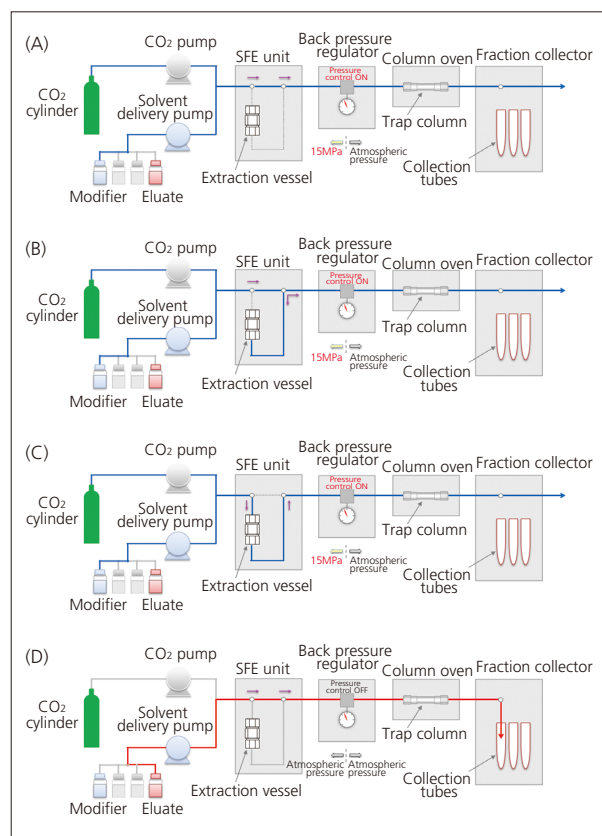


Fig. 1 Flow of Off-Line SFE Extraction

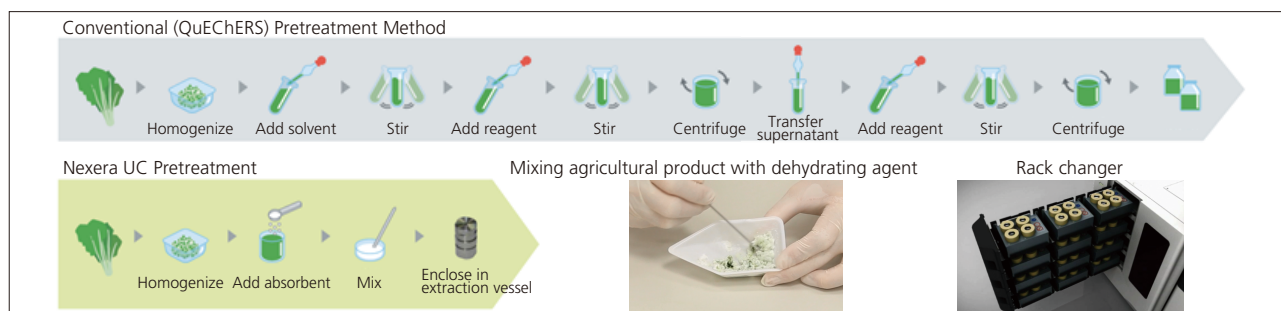


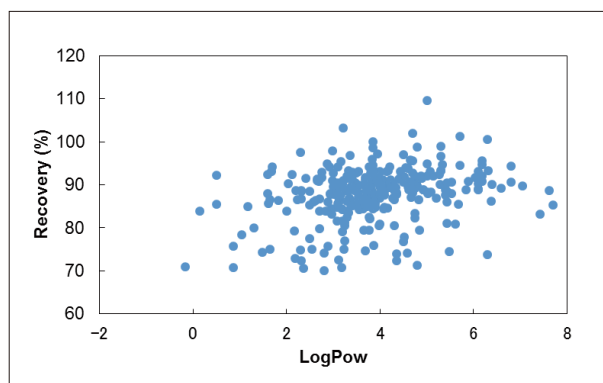
Fig. 2 Sample Preparation

**Table 1 Analytical Conditions**

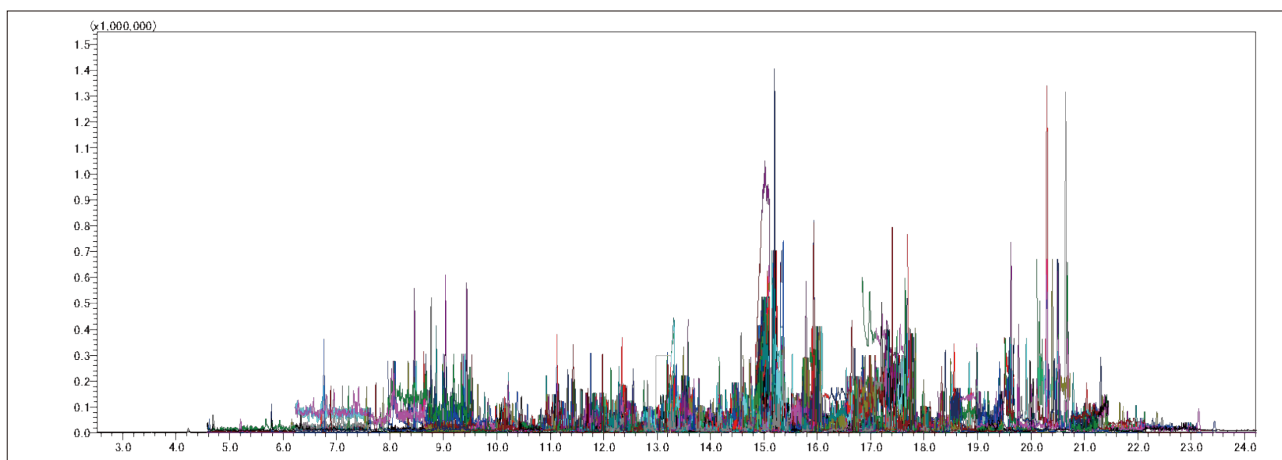
|   |   |
|---|---|
| [SFE] Nexera UC SFE System                                    | [GC-MS] GCMS-TQ8040   |
| Extraction : A) Supercritical fluid of CO <sub>2</sub>        | Column : Rxi®-5Sil MS 30 m × 0.25 mm I.D., df = 0.25 μm                             |
| Solvent : B) Methanol   | Column Temp. : 50 °C (1 min) → (25 °C/min) → 125 °C → (10 °C/min) → 300 °C (15 min) |
| Flowrate : 5 mL/min   | Carrier Gas : He (Constant linear velocity mode)                                    |
| Extraction : 8 min (Static mode → Dynamic mode)               | Linear Velocity : 47.2 cm/sec   |
| Extraction Vessel Temp. : 40 °C                               | Injection Mode : Splitless (Sampling time 1.00 min)                                 |
| BPR Pressure : 15 MPa   | High Press Inj. : 250 kPa (1.5 min)   |
| Trap Column : Shim-pack VP-ODS (50 mm L. × 4.6 mm I.D., 5 μm) | Injection Volume : 1 μL   |
| Elution Solvent : Acetone/Hexane = 50/50 (2 mL/min, 2 min)    | Interface Temp. : 250 °C  |
|   | Ion Source Temp. : 200 °C   |
|   | MS Mode : MRM   |
|   | Loop Time : 0.3 sec   |

**■ Analysis of Brown Rice**

A mixed standard solution of pesticides for GC/MS analysis (Hayashi Pure Chemical PL2005 Pesticide GC/MS Mix I-VI, 7) was added to pulverized brown rice to a concentration of 100 ng/g, and SFE was performed using the conditions shown in Table 1. The extraction liquid obtained was made up to 2 mL using eluate, and analyzed by GC/MS. The MRM chromatogram obtained from GC/MS analysis is shown in Fig. 4. Good repeatability (relative standard deviation of quantitation concentration <10 %) and recovery (70-120 %) were obtained for the 301 components. Repeatability and recovery for the 301 pesticides are shown in Table 2. This system uses a very simple pretreatment process, and can perform automated extraction from a single sample in approximately 30 minutes.



**Fig. 3 Recovery in Brown Rice Analysis**



**Fig. 4 MRM Chromatogram of Extraction Liquid from Brown Rice**

**Table 2 Repeatability and Recovery**

| Compounds        | Repeatability (%RSD, n = 6) | Recovery (%) | Compounds       | Repeatability (%RSD, n = 6) | Recovery (%) |
|------------------|-----------------------------|--------------|-----------------|-----------------------------|--------------|
| 2-Phenylphenol   | 3.8                         | 87.0         | Azinphos-ethyl  | 5.3                         | 84.3         |
| Acetochlor       | 5.9                         | 93.1         | Azinphos-methyl | 2.7                         | 83.1         |
| Acrinathrin-1    | 6.8                         | 73.8         | Benalaxyl       | 7.0                         | 84.9         |
| Acrinathrin-2    | 3.1                         | 100.6        | Benfluralin     | 5.2                         | 90.1         |
| Alachlor         | 3.6                         | 88.7         | Benfuresate     | 4.1                         | 91.5         |
| Allethrin-3,4    | 5.9                         | 102.0        | Benoxacor       | 3.2                         | 90.8         |
| Allidochlor      | 5.3                         | 86.4         | beta-BHC        | 5.3                         | 87.8         |
| alpha-BHC        | 4.6                         | 88.9         | beta-Endosulfan | 6.5                         | 90.7         |
| alpha-Endosulfan | 9.5                         | 98.7         | Bifenox         | 4.1                         | 84.5         |
| Ametryn          | 4.1                         | 86.3         | Bifenthrin      | 3.3                         | 89.2         |
| Anilofos         | 4.7                         | 86.3         | Biphenyl        | 3.5                         | 80.5         |
| Atrazine         | 4.8                         | 86.7         | Bromobutide     | 4.6                         | 90.4         |
| Azaconazole      | 5.5                         | 70.5         | Bromophos       | 5.4                         | 90.1         |
| Azamethiphos     | 9.9                         | 78.4         | Bromophos-ethyl | 6.0                         | 86.6         |

Table 2 Repeatability and Recovery (continued)

| Compounds           | Repeatability (%RSD, n = 6) | Recovery (%) | Compounds            | Repeatability (%RSD, n = 6) | Recovery (%) |
|---------------------|-----------------------------|--------------|----------------------|-----------------------------|--------------|
| Bromopropylate      | 4.1                         | 90.9         | Diphenamid           | 5.7                         | 79.3         |
| Bromuconazole-1     | 3.7                         | 80.5         | Diphenylamine        | 3.1                         | 91.5         |
| Bromuconazole-2     | 5.3                         | 77.1         | Disulfoton sulfone   | 5.2                         | 85.0         |
| Bupirimate          | 7.9                         | 86.8         | Ditalimfos           | 3.2                         | 90.1         |
| Buprofezin          | 6.6                         | 88.8         | Dithiopyr            | 5.1                         | 90.9         |
| Butachlor           | 6.4                         | 91.6         | Edifenphos           | 3.5                         | 95.9         |
| Butafenacil         | 4.4                         | 90.4         | Endosulfan sulfate   | 6.9                         | 95.4         |
| Butamifos           | 3.8                         | 90.1         | EPN                  | 3.8                         | 88.0         |
| Butylate            | 4.6                         | 84.7         | Epoxiconazole        | 3.7                         | 83.9         |
| Cadusafos           | 4.1                         | 88.1         | EPTC                 | 4.3                         | 81.6         |
| Cafenstrole         | 5.1                         | 91.1         | Esprocarb            | 2.7                         | 90.6         |
| Captan              | 9.1                         | 77.6         | Ethalfuralin         | 5.3                         | 93.3         |
| Carbofuran          | 4.7                         | 83.3         | Ethion               | 3.4                         | 93.1         |
| Carbophenothion     | 2.9                         | 91.5         | Ethofumesate         | 5.7                         | 91.4         |
| Carfentrazone-ethyl | 4.1                         | 96.8         | Ethoprophos          | 4.3                         | 91.0         |
| Chinomethionat      | 4.2                         | 82.1         | Etobenzanid          | 3.8                         | 86.6         |
| Chlormethoxyfen     | 5.8                         | 89.8         | Etofenprox           | 3.8                         | 89.7         |
| Chlorbenside        | 3.9                         | 81.1         | Etoazole             | 8.2                         | 87.9         |
| Chlorbufam          | 4.2                         | 84.7         | Etridiazole          | 3.8                         | 85.3         |
| Chlorethoxyfos      | 4.6                         | 90.3         | Etrimfos             | 2.9                         | 87.9         |
| Chlorfenapyr        | 7.5                         | 86.5         | Famoxadone           | 5.4                         | 71.2         |
| Chlorfenson         | 7.7                         | 91.4         | Fenamidone           | 5.7                         | 70.1         |
| Chlorfenvinphos-(E) | 4.4                         | 91.2         | Fenchlorphos         | 6.0                         | 92.1         |
| Chlorfenvinphos-(Z) | 6.5                         | 88.7         | Fenitrothion         | 6.9                         | 88.7         |
| Chlormephos         | 3.1                         | 89.6         | Fenothiocarb         | 5.4                         | 88.6         |
| Chlorobenzilate     | 3.6                         | 92.0         | Fenoxanil            | 6.2                         | 88.2         |
| Chloroneb           | 6.0                         | 95.0         | Fenoxaprop-ethyl     | 4.1                         | 90.5         |
| Chlorothalonil      | 5.3                         | 87.7         | Fenoxycarb           | 6.9                         | 84.4         |
| Chlorpropham        | 4.9                         | 88.5         | Fenpropathrin        | 3.7                         | 91.6         |
| Chlorpyrifos        | 6.2                         | 90.8         | Fenpropimorph        | 4.7                         | 76.8         |
| Chlorpyrifos-methyl | 5.1                         | 90.5         | Fenthion             | 3.6                         | 79.5         |
| Chlorthiophos-2     | 9.5                         | 88.4         | Fenvalerate-1        | 5.2                         | 88.4         |
| Chlorthiophos-3     | 2.8                         | 92.8         | Fenvalerate-2        | 4.2                         | 95.0         |
| Chlozolinate        | 7.8                         | 82.4         | Fipronil             | 8.3                         | 86.7         |
| Cinidon-ethyl       | 4.3                         | 88.8         | Flamprop-methyl      | 6.6                         | 85.7         |
| Cinmethylin         | 9.9                         | 94.5         | Fluacrypyrim         | 6.8                         | 97.0         |
| Clomazone           | 4.2                         | 88.6         | Flucythrinate-1      | 4.0                         | 92.8         |
| Clomeprop           | 3.3                         | 89.8         | Flucythrinate-2      | 3.7                         | 95.7         |
| Crimidine           | 6.0                         | 80.0         | Flufenpyr-ethyl      | 1.8                         | 98.0         |
| Cyanofenphos        | 4.7                         | 91.8         | Flumiclorac-pentyl   | 5.8                         | 91.8         |
| Cyanophos           | 5.0                         | 91.3         | Flumioxazin          | 9.4                         | 75.0         |
| Cyflufenamid        | 8.4                         | 89.6         | Fluquinconazole      | 4.3                         | 81.2         |
| Cyfluthrin-1        | 5.1                         | 95.6         | Flusilazole          | 5.5                         | 86.8         |
| Cyfluthrin-2        | 3.5                         | 94.6         | Fluthiacet-methyl    | 3.8                         | 79.5         |
| Cyfluthrin-3        | 4.9                         | 92.0         | Flutolanil           | 9.6                         | 87.8         |
| Cyfluthrin-4        | 6.0                         | 90.8         | Fluvalinate-1        | 2.6                         | 100.0        |
| Cyhalofop-butyl     | 4.2                         | 93.4         | Fluvalinate-2        | 2.6                         | 98.6         |
| Cyhalothrin-1       | 9.1                         | 90.6         | Folpet               | 5.3                         | 87.7         |
| Cyhalothrin-2       | 4.5                         | 94.4         | Fonofos              | 3.8                         | 91.7         |
| Cypermethrin-1      | 2.8                         | 99.0         | Formothion           | 5.3                         | 74.4         |
| Cypermethrin-2      | 3.7                         | 96.6         | Fosthiazate-2        | 9.6                         | 93.2         |
| Cypermethrin-3      | 3.7                         | 93.2         | Furilazole           | 3.3                         | 92.4         |
| Cypermethrin-4      | 8.4                         | 93.2         | gamma-BHC            | 4.1                         | 88.7         |
| Cyprodinil          | 4.0                         | 80.9         | Halfenprox           | 2.3                         | 85.4         |
| delta-BHC           | 2.2                         | 88.2         | Hexaconazole         | 8.9                         | 85.6         |
| Deltamethrin-2      | 3.7                         | 103.2        | Indanofan            | 7.9                         | 86.5         |
| Dialifos            | 3.2                         | 91.4         | Indoxacarb           | 3.7                         | 95.7         |
| Di-allate-1         | 2.5                         | 91.5         | Iprobenfos           | 4.4                         | 89.5         |
| Di-allate-2         | 4.7                         | 92.0         | Iprodione            | 2.5                         | 92.7         |
| Diazinon            | 7.8                         | 90.0         | Iprodione metabolite | 3.1                         | 106.2        |
| Dichlobenil         | 4.0                         | 79.8         | Isazofos             | 3.7                         | 94.2         |
| Dichlofenthion      | 5.2                         | 92.1         | Isocarbophos         | 6.6                         | 84.0         |
| Dichlofluanid       | 3.3                         | 87.2         | Isofenphos           | 3.2                         | 89.0         |
| Dichlorvos          | 3.2                         | 83.9         | Isofenphos oxon      | 5.2                         | 84.5         |
| Diclobutrazol       | 5.2                         | 87.0         | Isoprocarb           | 4.5                         | 86.6         |
| Diclocymet-1        | 4.3                         | 83.4         | Isoprothiolane       | 7.5                         | 86.1         |
| Diclocymet-2        | 5.1                         | 82.2         | Isxadifen-ethyl      | 5.0                         | 90.5         |
| Diclofop-methyl     | 4.4                         | 91.0         | Isoxathion           | 6.7                         | 93.2         |
| Diethofencarb       | 4.8                         | 83.8         | Kresoxim-methyl      | 7.0                         | 89.7         |
| Difenoconazole-1    | 5.5                         | 74.0         | Leptophos            | 3.5                         | 93.3         |
| Difenoconazole-2    | 5.2                         | 72.4         | Malathion            | 3.2                         | 93.0         |
| Diflufenican        | 4.4                         | 94.3         | MCPB-ethyl           | 3.5                         | 90.3         |
| Dimepiperate        | 2.5                         | 87.8         | Mecarbam             | 8.4                         | 97.6         |
| Dimethametryn       | 6.4                         | 84.8         | Mefenacet            | 4.5                         | 75.1         |
| Dimethenamid        | 5.4                         | 88.8         | Mefenpyr-diethyl     | 5.0                         | 90.4         |
| Dimethipin          | 9.9                         | 70.9         | Mepronil             | 4.2                         | 79.5         |
| Dimethylvinphos-(E) | 4.5                         | 86.8         | Metalaxyl            | 7.0                         | 86.6         |
| Dimethylvinphos-(Z) | 4.9                         | 86.1         | Methacrifos          | 5.9                         | 92.3         |
| Diniconazole        | 2.3                         | 80.6         | Methidathion         | 4.5                         | 86.0         |
| Dioxabenzofos       | 4.4                         | 91.5         | Methoprene           | 8.8                         | 109.6        |
| Dioxathion          | 5.4                         | 88.6         | Methoxychlor         | 3.1                         | 90.6         |
| Dioxathion deg.     | 4.4                         | 86.1         | Metolachlor          | 2.9                         | 91.1         |

Table 2 Repeatability and Recovery (continued)

| Compounds                | Repeatability (%RSD, n = 6) | Recovery (%) | Compounds         | Repeatability (%RSD, n = 6) | Recovery (%) |
|--------------------------|-----------------------------|--------------|-------------------|-----------------------------|--------------|
| Metominostrobin-(E)      | 9.6                         | 72.4         | Simazine          | 5.2                         | 74.9         |
| Metribuzin               | 6.5                         | 75.1         | Simeconazole      | 6.1                         | 79.1         |
| Mevinphos-1              | 9.9                         | 92.3         | Simetryn          | 5.0                         | 74.1         |
| Mevinphos-2              | 6.0                         | 85.4         | Spirodiclofen     | 4.6                         | 94.1         |
| Molinate                 | 3.8                         | 86.0         | Sulfotep          | 3.9                         | 92.9         |
| Myclobutanil             | 5.9                         | 75.7         | Sulprofos         | 4.8                         | 74.5         |
| Naled                    | 6.1                         | 72.8         | Swep              | 5.3                         | 83.6         |
| Nitralin                 | 4.5                         | 94.2         | Tebufenpyrad      | 3.6                         | 88.8         |
| Nitrofen                 | 8.1                         | 88.9         | Tebupirimfos      | 4.6                         | 89.4         |
| Nitrothal-isopropyl      | 2.4                         | 90.2         | Tecnazene         | 3.1                         | 89.5         |
| Oxabetrinil              | 3.4                         | 91.7         | Tefluthrin        | 4.5                         | 90.1         |
| Oxadiazon                | 3.9                         | 94.7         | Terbucarb         | 4.0                         | 87.6         |
| Oxpoconazole             | 6.4                         | 74.7         | Terbufos          | 3.8                         | 77.9         |
| Oxpoconazole-formyl deg. | 9.9                         | 88.9         | Terbutryn         | 4.5                         | 86.0         |
| Oxyfluorfen              | 8.9                         | 88.3         | Tetrachlorvinphos | 3.2                         | 93.0         |
| Paclobutrazol            | 7.5                         | 72.6         | Tetraconazole     | 7.8                         | 84.3         |
| Parathion                | 6.3                         | 90.1         | Tetradifon        | 5.9                         | 89.5         |
| Parathion-methyl         | 5.1                         | 90.4         | Tetramethrin-1    | 6.9                         | 93.8         |
| Penconazole              | 4.7                         | 85.0         | Tetramethrin-2    | 4.3                         | 90.9         |
| Pendimethalin            | 5.1                         | 86.9         | Thenylchlor       | 3.5                         | 87.3         |
| Pentoxazone              | 4.2                         | 95.6         | Thifluzamide      | 5.9                         | 84.6         |
| Permethrin-1             | 4.8                         | 89.0         | Thiobencarb       | 3.5                         | 85.6         |
| Permethrin-2             | 4.0                         | 88.8         | Tolclofos-methyl  | 3.9                         | 90.6         |
| Phenothrin-1             | 7.4                         | 93.1         | Tolfenpyrad       | 3.6                         | 81.0         |
| Phenothrin-2             | 2.5                         | 90.2         | Tolyfluanid       | 5.8                         | 91.1         |
| Phenthoate               | 2.4                         | 91.7         | Triadimefon       | 3.7                         | 88.3         |
| Phorate                  | 4.1                         | 75.9         | Triadimenol-1     | 6.2                         | 70.8         |
| Phosalone                | 3.5                         | 88.1         | Tri-allate        | 5.3                         | 91.2         |
| Phosmet                  | 4.2                         | 84.5         | Triazophos        | 4.7                         | 89.9         |
| Phosphamidon-1           | 8.6                         | 75.8         | Tribufos          | 6.2                         | 90.6         |
| Phosphamidon-2           | 6.6                         | 70.8         | Trichlamide       | 5.2                         | 85.3         |
| Picolinafen              | 4.0                         | 90.4         | Trifloxystrobin   | 5.9                         | 90.7         |
| Piperonyl butoxide       | 3.8                         | 89.2         | Trifluralin       | 3.2                         | 92.5         |
| Piperophos               | 3.5                         | 88.9         | Vinclozolin       | 4.2                         | 89.6         |
| Pirimiphos-methyl        | 5.7                         | 90.8         | XMC               | 3.9                         | 86.5         |
| Pretilachlor             | 5.6                         | 89.8         | Xyllycarb         | 4.5                         | 85.3         |
| Procymidone              | 7.0                         | 91.6         | Zoxamide          | 3.6                         | 82.6         |
| Profenofos               | 5.6                         | 94.1         |                   |                             |              |
| Prohydrojasmon-1         | 5.5                         | 87.7         |                   |                             |              |
| Prohydrojasmon-2         | 8.7                         | 88.6         |                   |                             |              |
| Prometryn                | 3.0                         | 86.8         |                   |                             |              |
| Propachlor               | 4.4                         | 88.0         |                   |                             |              |
| Propargite-1             | 9.3                         | 101.3        |                   |                             |              |
| Propargite-2             | 9.5                         | 94.5         |                   |                             |              |
| Propazine                | 4.0                         | 97.1         |                   |                             |              |
| Propiconazole-1          | 6.7                         | 89.4         |                   |                             |              |
| Propiconazole-2          | 3.2                         | 88.3         |                   |                             |              |
| Propoxur                 | 5.3                         | 83.9         |                   |                             |              |
| Propyzamide              | 4.2                         | 81.6         |                   |                             |              |
| Prothiofos               | 4.0                         | 85.5         |                   |                             |              |
| Pyraclufos               | 5.1                         | 94.1         |                   |                             |              |
| Pyraclostrobin           | 4.7                         | 93.1         |                   |                             |              |
| Pyraflufen-ethyl         | 4.7                         | 92.7         |                   |                             |              |
| Pyrazophos               | 4.2                         | 92.8         |                   |                             |              |
| Pyrazoxyfen              | 9.4                         | 91.2         |                   |                             |              |
| Pyributicarb             | 3.1                         | 88.1         |                   |                             |              |
| Pyridaben                | 3.1                         | 86.1         |                   |                             |              |
| Pyridaphenthion          | 5.4                         | 84.2         |                   |                             |              |
| Pyrifenox-(E)            | 5.9                         | 85.2         |                   |                             |              |
| Pyrifenox-(Z)            | 7.3                         | 92.9         |                   |                             |              |
| Pyrimethanil             | 6.0                         | 83.9         |                   |                             |              |
| Pyrimidifen              | 4.9                         | 74.2         |                   |                             |              |
| Pyriminobac-methyl-(E)   | 3.9                         | 88.6         |                   |                             |              |
| Pyriminobac-methyl-(Z)   | 5.2                         | 88.6         |                   |                             |              |
| Pyriproxyfen             | 5.7                         | 92.1         |                   |                             |              |
| Quinalphos               | 3.3                         | 93.2         |                   |                             |              |
| Quinoxifen               | 3.2                         | 87.1         |                   |                             |              |
| Quintozene               | 6.0                         | 90.3         |                   |                             |              |
| Quisalofop-ethyl         | 3.0                         | 86.9         |                   |                             |              |
| Resmethrin-1             | 6.2                         | 88.5         |                   |                             |              |
| Resmethrin-2             | 3.3                         | 86.1         |                   |                             |              |
| Silafluofen              | 3.7                         | 88.6         |                   |                             |              |

