

Simple and Efficient Multiresidue Screening Method for Analysis of Nine Halogen-Containing Pesticides on Peppers and Cucumbers by GLC-ECD[†]

A. Valverde García,* E. González Pradas, J. Martínez Vidal, and A. Agüera López

Departamentos de Química Inorgánica y Química Analítica, Facultad de Ciencias Experimentales de Almería, Universidad de Granada, 04071 Almería, Spain

A simple and efficient multiresidue extraction procedure using ethyl acetate and sodium sulfate has been applied to the analysis of chlorpyrifos, dichlofluanid, dicloran, endosulfan I, endosulfan II, endosulfan sulfate, γ -HCH, procymidone, and vinclozolin residues in peppers and cucumbers. A short-column Florisil cleanup preceded the analysis by capillary gas-liquid chromatography with electron capture detection. Recoveries of each pesticide have been calculated in both vegetables at three different fortification levels. Recoveries are greater than 80% in all cases, except for vinclozolin where recoveries greater than 74% were obtained. The limit of quantification of the analytical method has been estimated as 0.1 mg/kg for the nine pesticides in both matrices. The proposed method has been applied to the analysis of 497 samples of peppers and cucumbers grown in Almería's greenhouses (Spain).

INTRODUCTION

The constantly expanding use of pesticides on food crops continually accentuates the need for more rapid, precise, and sensitive methods for the determination of pesticide residues remaining in foods.

Several multiresidue extraction methods for analysis of pesticide residues in vegetables have been used. The more important (McMahon and Burke, 1987) are the Mills method (Mills et al., 1963; AOAC, 1984), which uses extraction with acetonitrile and partition with petroleum ether, and the Luke method (Luke et al., 1975, 1981; AOAC, 1990), which uses extraction with acetone and partition with petroleum ether and dichloromethane. Nevertheless, the Mills multiresidue extraction method is in general limited to relatively nonpolar compounds (McMahon and Burke, 1978), and the Luke method and its modifications (Andersson and Ohlim, 1986), which recover a greater number of polar pesticides, are in general expensive and tedious when they are applied to a large number of samples.

Ethyl acetate and sodium sulfate were used by Leary as a specific extraction method for residues of methamidophos in vegetables, using GLC with thermionic detection and carrying out a previous cleanup on silica gel columns (Leary, 1971). Nowadays ethyl acetate and sodium sulfate are used for analysis of methamidophos in crops by the laboratories of the National Food Administration (Uppsala, Sweden) and the Food Production and Inspection Branch, Agriculture Canada (Ottawa, Canada), using GLC with flame photometric detection (FPD) in phosphorus mode without a previous cleanup step (Andersson and Ohlim, 1986; Food Production and Inspection Branch, 1984). These reagents have also been used by the present authors for analysis of several pesticide residues in vegetables, without a cleanup step using GLC with nitrogen-phosphorus detection (NPD) (Martínez et al., 1990). Likewise, ethyl acetate and sodium sulfate are also used in the extraction step of the "sweep codistillation method", described in the AOAC multiple-residue methods for organophosphorus pesticides, using GLC with KCl thermionic detection (KCITD) and a previous "sweep co-

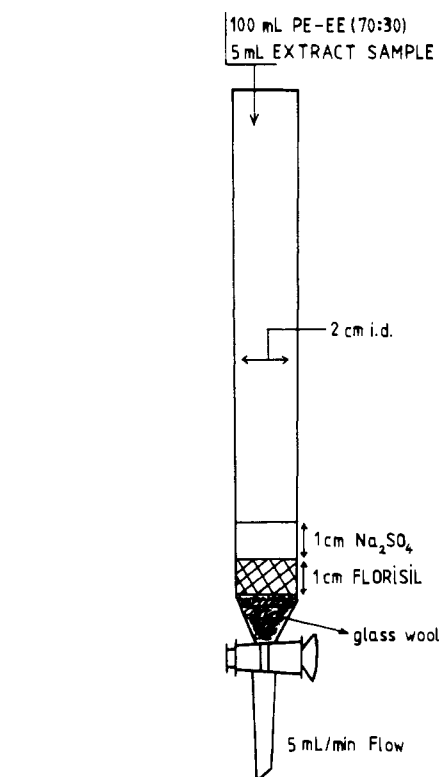


Figure 1. Cleanup column.

distillation cleanup" (AOAC, 1990). However, these reagents have not been used yet to analyze pesticide residues with electron capture detection.

The objective of the present research was to study the use of a rapid one-step extraction method, which only uses extraction with ethyl acetate and sodium sulfate, as a screening method for analysis of chlorpyrifos, dichlofluanid, dicloran, endosulfan I, endosulfan II, endosulfan sulfate, γ -HCH, procymidone, and vinclozolin residues in peppers and cucumbers by gas-liquid chromatography with electron capture detection (GLC-ECD) and a cleanup step on short Florisil columns.

[†]This work was supported by the CICYT, Project ALI89-0020.

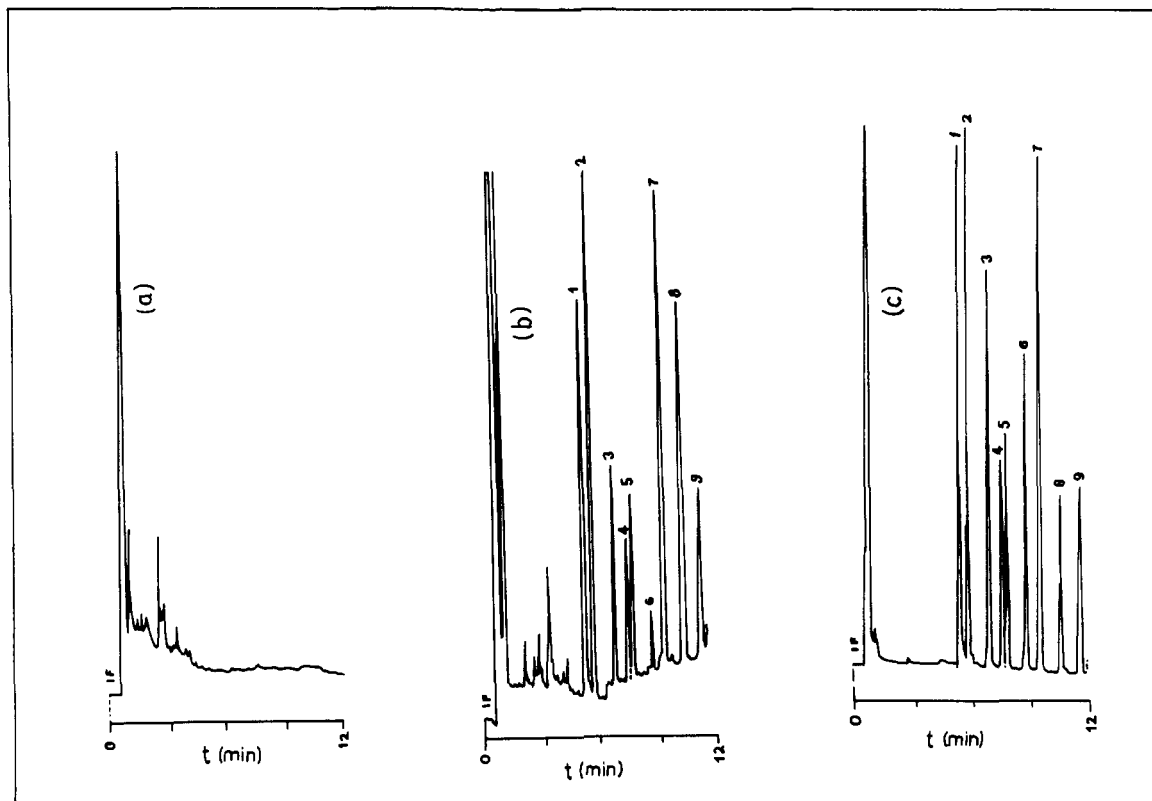


Figure 2. Chromatograms obtained for (a) a blank sample of pepper, (b) a spiked sample of pepper at a fortification level of 0.2 mg/kg (equivalent to 1.0 mg/L in the extract) of dicloran, γ -HCH, vinclozolin, dichlofluanid, chlorpyrifos, procymidone, endosulfan I, endosulfan II, and endosulfan sulfate, and (c) the pesticide standard solution (1.04 mg/L dicloran, 1.00 mg/L γ -HCH, 1.18 mg/L vinclozolin, 1.01 mg/L dichlofluanid, 1.05 mg/L chlorpyrifos, 4.93 mg/L procymidone, 1.04 mg/L endosulfan I, 0.52 mg/L endosulfan II, and 1.01 mg/L endosulfan sulfate). (1) Dicloran; (2) γ -HCH; (3) vinclozolin; (4) dichlofluanid; (5) chlorpyrifos; (6) procymidone; (7) endosulfan I; (8) endosulfan II; (9) endosulfan sulfate.

Table I. Retention Times of Pesticides on GLC Wide Bore Capillary Column (HP-1)^a

peak	pesticide	RT, min
1	dicloran	5.46
2	γ -HCH	5.92
3	vinclozolin	7.12
4	dichlofluanid	7.83
5	chlorpyrifos	8.09
6	procymidone	9.11
7	endosulfan I	9.79
8	endosulfan II	10.78
9	endosulfan sulfate	11.74

^a See text for GLC conditions and column characteristics.

EXPERIMENTAL PROCEDURES

Reagents and Apparatus. (a) Solvents were ethyl acetate, Scharlau (99.9%), pesticide residue grade, and petroleum ether, Scharlau (40–60 °C), pesticide residue grade.

(b) Anhydrous sodium sulfate, Merck AR (99.5%), 12–60 mesh, was heated at 300 °C overnight.

(c) Florisil, 60–100 mesh, Baker analyzed reagent, was activated at 650 °C and reheated at 130 °C for 5 h before use.

(d) Pesticide standards, Pestanal (purity >98%), of chlorpyrifos, dichlofluanid, dicloran, endosulfan I, endosulfan II, endosulfan sulfate, γ -HCH, procymidone, and vinclozolin were used.

(e) *Pesticide Standard Solution.* Petroleum ether solutions of chlorpyrifos, dichlofluanid, dicloran, endosulfan I, endosulfan II, endosulfan sulfate, γ -HCH, procymidone, and vinclozolin were used. Pesticide concentrations are indicated in Figure 2c caption.

(f) The rotary vacuum evaporator was a Heidolph W 2000.

(g) An Omni mixer having a 400-mL maximum capacity was used.

(h) The gas chromatograph was a Hewlett-Packard Model 5890A with electron capture detector (ECD), equipped with a wide bore fused silica capillary column (HP-1) containing 100%

dimethylpolysiloxane as nonpolar stationary phase (30 m length, 0.53 mm i.d. and 2.65- μ m film thickness). The following operating conditions were used: carrier gas flow (nitrogen), 10 mL/min; auxiliary gas flow (nitrogen), 50 mL/min; injector temperature, 260 °C; detector temperature, 300 °C; injection volume, 1 μ L; oven temperature program

160 °C (1 min) $\xrightarrow{20\text{ }^\circ\text{C}/\text{min}}$ 220 °C (2 min) $\xrightarrow{10\text{ }^\circ\text{C}/\text{min}}$ 260 °C (3 min)

Preparation of Sample. Macerate the sample in a food chopper. Weigh 50 g of chopped sample into a high-speed blender jar and add 80 g of anhydrous sodium sulfate. Mix thoroughly, add 100 mL of ethyl acetate, and blend the mixture for 5 min. Filter the supernatant liquid by suction through a 12-cm Büchner funnel with Whatman Ashless 40 filter paper and a layer of 20 g of anhydrous sodium sulfate. Repeat the extraction and filtration steps two more times, adding the plant tissue to the funnel in the last filtration step. Rinse the filter with 50 mL of ethyl acetate and evaporate the combined extracts to dryness on a vacuum rotary evaporator using a 40–60 °C water bath. Dissolve the residue in exactly 10 mL of a 9:1 petroleum ether-acetone mixture.

Cleanup. The following cleanup process was applied before the analysis: Place 5 mL of sample extract on a glass column (2 cm i.d.) containing about 1.1 g of Florisil (1-cm length) and about 4.5 g of anhydrous sodium sulfate (1-cm length) and previously saturated with petroleum ether (see Figure 1). Elute the pesticide residues with 100 mL of petroleum ether solution containing 30% v/v of diethyl ether (5 mL/min flow), concentrate the eluate to 1–2 mL using a vacuum rotary evaporator (40–60 °C water bath), and dilute the extract to exactly 5 mL with petroleum ether in a volumetric flask.

Gas-Liquid Chromatography. Inject 1 μ L of sample extract after the cleanup process (5-mg sample equivalent) into the capillary column HP-1 using the operating conditions indicated above. Compare peaks with standard pesticide solution and quantitate using external standard method.

Table II. Pesticide Recoveries in Peppers Using the Proposed Procedure

pesticide	recovery, ^a % (range), at fortification level of			recovery, ^b % (range)
	0.1 mg/kg	0.2 mg/kg	0.4 mg/kg	
dicloran	98.3 (92.7–107.0)	91.5 (86.4–95.3)	96.1 (91.0–99.3)	95.3 (86.4–107.0)
γ -HCH	89.6 (82.0–93.5)	90.4 (81.3–101.3)	94.8 (87.1–103.6)	91.6 (81.3–103.6)
vinclozolin	79.6 (76.1–81.5)	77.7 (70.6–83.9)	85.2 (79.4–90.1)	80.8 (70.6–90.1)
dichlofluanid	84.3 (93.3–99.8)	91.6 (87.5–97.0)	98.7 (89.8–103.8)	94.9 (83.3–103.8)
chlorpyrifos	99.3 (95.1–106.5)	102.6 (97.4–110.6)	93.4 (91.3–94.7)	98.4 (91.3–110.6)
procymidone	90.8 (83.1–98.1)	97.2 (91.0–100.1)	85.5 (79.8–94.9)	91.2 (79.8–100.1)
endosulfan I	105.5 (101.7–109.3)	96.9 (94.1–98.9)	103.1 (95.0–109.8)	101.8 (94.1–109.8)
endosulfan II	97.0 (96.0–97.6)	95.6 (90.0–98.7)	93.8 (89.8–97.3)	95.5 (90.0–98.7)
endosulfan sulfate	89.4 (86.4–94.2)	99.1 (92.6–111.8)	89.6 (82.4–96.2)	92.7 (82.4–111.8)

^a Average of three replicates. ^b Average of the nine replicates (without taking into account fortification levels).

Table III. Pesticide Recoveries in Cucumbers Using the Proposed Procedure

pesticide	recovery, ^a % (range), at fortification level of			recovery, ^b % (range)
	0.1 mg/kg	0.2 mg/kg	0.4 mg/kg	
dicloran	97.1 (89.9–103.0)	88.5 (82.1–93.0)	95.5 (92.3–98.4)	93.7 (82.1–103.0)
γ -HCH	111.1 (100.1–124.7)	81.1 (70.1–87.0)	96.6 (87.1–103.0)	96.3 (70.1–124.7)
vinclozolin	74.1 (70.6–77.2)	84.2 (75.4–91.3)	84.0 (82.3–86.3)	80.8 (70.6–91.3)
dichlofluanid	92.7 (81.8–99.2)	93.8 (79.6–102.1)	90.6 (79.8–97.4)	92.4 (79.6–102.1)
chlorpyrifos	95.2 (86.4–107.4)	97.7 (87.3–103.5)	94.3 (88.5–98.3)	95.7 (86.4–107.4)
procymidone	95.4 (84.4–101.8)	85.1 (79.3–95.2)	92.8 (87.2–95.9)	91.1 (79.3–101.8)
endosulfan I	108.1 (96.9–117.5)	89.7 (86.1–92.2)	96.4 (90.2–99.7)	98.1 (86.1–117.5)
endosulfan II	94.2 (83.1–102.8)	90.8 (86.0–95.0)	89.2 (82.1–94.1)	91.4 (82.1–102.8)
endosulfan sulfate	97.9 (88.0–105.8)	92.8 (83.9–103.9)	94.1 (83.2–99.6)	94.9 (83.2–105.8)

^a Average of three replicates. ^b Average of the nine replicates (without taking into account fortification levels).

Recoveries. Pesticide recovery study was carried out by fortifying fresh pepper and cucumber samples grown in the Research Agricultural Center "Las Palmerillas de la Caja Rural de Almeria", which were not treated with the pesticides studied.

Since all of the pesticides analyzed according to this procedure are administered by foliar application and are nonsystemic (except procymidone, a fungicide with a moderate systemic activity) (Worthing, 1991), most of their residues remain on the plant surfaces. In addition, these pesticides are practically insoluble in water (except vinclozolin, which is very slightly soluble). So, to achieve a reasonable simulation of the true situation, the fortification process of plant tissue was carried out as follows: Ten milliliters of a petroleum ether solution containing chlorpyrifos, dichlofluanid, dicloran, endosulfan I, endosulfan II, endosulfan sulfate, γ -HCH, procymidone, and vinclozolin was added to 50 g of chopped sample in a high-speed blender jar. After petroleum ether evaporation by air stream, the sample (with the pesticide residue placed on the top of the plant tissue) was homogenized for 2 min. After 30 min, the sample was again homogenized for 1 min and immediately analyzed by application of the previously described method.

Recoveries were estimated in peppers and cucumbers at three different fortification levels for all pesticides, 0.1, 0.2, and 0.4 mg/kg, values corresponding to a concentration of each pesticide in the added solution of 0.5, 1.0, and 2 mg/L, respectively. Three replicates at each fortification level and three check samples of both vegetables were analyzed.

RESULTS AND DISCUSSION

Figure 2c shows the chromatogram obtained by gas-liquid chromatography with the HP-1 column corresponding to the pesticide standard solution, and Table I shows the retention time values of the pesticides studied.

Detector response was tested between 0.1 and 2.0 ng for all of the pesticides except for procymidone, which was tested between 0.5 and 5.0 ng, this response being linear for the ranges studied. The sensitivity of the ECD detector to pesticides in the standard solution was about 2×10^6 (peak area units per nanogram) except for procymidone, which was 5 times lower. The limit of quantification (LOQ) of the analytical method has been estimated as 0.1 mg/kg for all pesticides studied (the lowest concentration actually tested and validated). However, we estimate that it appears to be <0.1 ppm.

Parts a and b of Figure 2 show the chromatograms corresponding to a blank sample of pepper and a spiked sample of pepper at a fortification level of 0.2 mg/kg for each pesticide studied, respectively. The chromatograms obtained for the same type of check samples were not exactly the same. This may indicate that the method used cannot extract the plant coextractives from the matrix in a very reproducible manner. However, in all cases coextractive peaks did not interfere with the standard peaks.

Tables II and III show average recoveries obtained (for each fortification level) for the pesticides added to peppers and cucumbers, respectively, using the procedure described above. Tables II and III also show the average values corresponding to the percentages obtained for the nine recovery tests for each pesticide in peppers and cucumbers, respectively (without taking into account the different fortification levels); these values are greater than 80% in all cases. Values of the recovery ranges are also given in Tables II and III, the upper values, in the majority of cases, being far above 100%. This may be due to adsorption of the injected standards in the injector or on column, while the coextractives in the sample prevent adsorption. Recoveries above 100% for other pesticide residues in vegetables have been reported by several authors (Mills et al., 1963; Luke et al., 1981; Andersson and Ohlim, 1986), who have offered a similar rationale for high recoveries.

We may compare our results with those given in the review by Ambrus and Thier (1986), where the applicability of seven well-established multiresidue procedures is critically assessed, including, of course, the Mills and Luke methods. There it can be seen that endosulfan I, endosulfan II, endosulfan sulfate, chlorpyrifos, and γ -HCH are well recovered (>70%) from vegetables when both the Mills and the Luke methods are used, while dichlofluanid and vinclozolin are well recovered with the Luke method but no data are given for the Mills method. Dicloran is also well recovered when the Luke method is used, but the Mills method gave poor recoveries (40–70%). Finally, there also are no data for procymidone. However, two papers report the complete recovery of this pesticide by application of the Luke extraction method to different fruits and vegetables at fortification levels ranging between 0.3 and 1.0 mg/kg (Andersson and Ohlim, 1986; González et al., 1989).

The worst results have been found for vinclozolin, for which average recoveries between 74.1 and 84.2% have been obtained. However, these values are comparable with those given by Barbina-Taccheo et al. (1984) in a multiresidue method for five fungicides on apples and pears where vinclozolin recoveries ranged between 70.0 and 89.2% for fortification levels ranging between 0.1 and 0.7 mg/kg.

We may conclude that the pesticide recoveries obtained by applying the proposed screening method are, without taking into account the type of sample and the fortification levels, comparable with those obtained by the multiresidue methods normally used to analyze these pesticides.

The proposed screening method was applied to the analysis of 306 samples of peppers and 191 samples of cucumbers grown in Almería's greenhouses from October 1988 to January 1989 (Agüera-López, 1989). The results obtained show that some residues of the pesticides were detected in 63.7% of the peppers and 31.9% of the cucumbers; 4% of the samples exceeded the maximum residue levels (MRL) established by the Spanish legislation.

ACKNOWLEDGMENT

We thank M. García Contreras (COEXPHAL, Almería, Spain) for his collaboration in this work.

LITERATURE CITED

- Agüera-Lopez, A. In *Tesina de Licenciatura*; Facultad de Ciencias Químicas de la Universidad de Granada: Granada, 1989.
- Ambrus, A.; Thier, H. P. Application of Multiresidue Procedures in Pesticides Residues Analysis. *Pure Appl. Chem.* 1986, 58, 1035.
- Andersson, A.; Ohlim, B. A Capillary Gas Chromatographic Multiresidue Method for Determination of Pesticides in Fruits and Vegetables. *Vaar Foeda* 1986, 38, 79.
- AOAC. *Official Methods of Analysis*, 14th ed.; Williams, S., Ed.; AOAC: Arlington, VA, 1984.
- AOAC. *Official Methods of Analysis*, 15th ed.; Williams, S., Ed.; AOAC: Arlington, VA, 1990.
- Barbina-Taccheo, M.; Spessotto, C.; Bresin, B.; Bagarolo, L. Multiresidue Method for Captan, Folpet, Captafol, Vinclozolin and Iprodione on Italian Apples and Pears by Capillary Gas-Liquid Chromatography with Electron-Capture Detection. *Pestic. Sci.* 1984, 15, 612.
- Food Production and Inspection Branch, Agriculture Canada. *Laboratory Manual for Pesticide Residue Analysis in Agricultural Products*; Canada Government: Ottawa, 1984.
- Gonzalez, E.; Martinez, J. L.; Valverde, A.; Villafranca, M.; Castillo, J. Residue Levels of Procymidone and Pyrazophos in Beans Grown in Greenhouses: Influence of the Plantation Density and Crop Distribution. *Z. Anal. Chem.* 1989, 334, 702.
- Leary, J. B. Gas Chromatographic Determination of Monitor (O,S-Dimethyl Phosphoramidothioate) Residues in Crops. *J. Assoc. Off. Anal. Chem.* 1971, 54, 1396.
- Luke, M. A.; Froberg, J. E.; Masumoto, H. T. Extraction and Cleanup of Organochlorine, Organophosphate, Organonitrogen, and Hydrocarbon Pesticides in Produce for Determination by Gas-Liquid Chromatography. *J. Assoc. Off. Anal. Chem.* 1975, 58, 1020.
- Luke, M. A.; Froberg, J. E.; Dosse, G. M.; Masumoto, H. T. Improved Multiresidue Gas Chromatographic Determination of Organophosphorus, Organonitrogen and Organohalogen Pesticides in Produce, Using Flame Photometric and Electrolytic Conductivity Detectors. *J. Assoc. Off. Anal. Chem.* 1981, 64, 1187.
- Martinez, J.; Valverde, A.; Gonzalez, E.; Roldan, E. *An. Quim.* 1990, 87, 248.
- McMahon, B.; Burke, J. A. Analytical Behavior Data for Chemicals Determined Using AOAC Multiresidue Methodology for Pesticide Residues in Food. *J. Assoc. Off. Anal. Chem.* 1978, 61, 640.
- McMahon, B.; Burke, J. A. Expanding and Tracking the Capabilities of Pesticide Multiresidue Methodology Used in the Food and Drug Administration's. Pesticide Monitoring Programs. *J. Assoc. Off. Anal. Chem.* 1987, 70, 1072.
- Mills, P. A.; Onley, J. H.; Gaither, R. A. Rapid Method for Chlorinated Pesticide Residues in Nonfatty Foods. *J. Assoc. Off. Anal. Chem.* 1963, 46, 186.
- Worthing, C. R., Ed. *The Pesticide Manual. A World Compendium*; The British Crop Protection Council: Croydon, U.K., 1991.

Received for review December 3, 1990. Revised manuscript received May 7, 1991. Accepted September 24, 1991.

Registry No. γ -HCH, 58-89-9; dichloran, 99-30-9; vinclozolin, 50471-44-8; dichlofluanid, 1085-98-9; chlorpyrifos, 2921-88-2; procymidone, 32809-16-8; endosulfan (I), 959-98-8; endosulfan (II), 33213-65-9; endosulfan sulfate, 1081-07-8.