RESIDUES AND TRACE ELEMENTS

Interlaboratory Study of a Multiresidue Gas Chromatographic Method for Determination of Organochlorine and Pyrethroid Pesticides and Polychlorobiphenyls in Milk, Fish, Eggs, and Beef Fat

FRANÇOIS BORDET,[1] DARY INTHAVONG,[1] and JEAN-MARC FREMY
Agence Française de Sécurité Sanitaire des Aliments, Unité Contaminants de l'Environnement, 10 Rue Pierre Curie, 94704, Maisons-Alfort, Paris, France

Collaborators: D. Aspe; T. Durand; M. Ducher; N. Fontayne; E. Grignon; C. Herbreteau; S. Hulot; C. Le Bigot; E. Marengue; V. Martin; S. Ranneaud; L. Sartre; B. Vouillon

An interlaboratory study was conducted to validate a gas chromatographic (GC) method for determination of 21 organochlorine pesticides, 6 pyrethroid pesticides, and 7 polychlorobiphenyl (PCB) congeners in milk, beef fat, fish, and eggs. The method was performed at low contamination levels, which represent relevant contents in food, and is an extension of the European standard (method NF-EN-1528, Parts 1–4). It enlarges the applicable scope of the reference EN method to pyrethroid pesticides and proposes the use of solid-phase extraction (SPE) as a cleanup procedure. Cryogenic extraction was made, and SPE cleanup was performed with 2 successive SPE cartridges: C_{18} and Florisil®. After injection of the purified extract onto a GC column, residues were measured by electron capture detection. Food samples (liquid milk, beef fat, mixed fish, and mixed eggs) were prepared, tested for homogeneity, and sent to 17 laboratories in France. Test portions were spiked with 27 pesticides and 7 PCBs at levels from 26 to 45, 4 to 27, 31 to 67, and 19 to 127 ng/g into milk, eggs, fish, and fat, respectively. Based on results for spiked samples, the relative standard deviation for repeatability ranged from 1.5 to 6.8% in milk, 3 to 39% in eggs, 4.5 to 12.2% in fish, and 7 to 13% in fat. The relative standard deviation for reproducibility ranged from 33 to 50% in milk, 29 to 59% in eggs, 31 to 57% in fish, and 30 to 62% in fat. This method showed acceptable intra- and interlaboratory precision data, as corroborated by HORRAT values at low levels of pesticide and PCB contamination. The statistical evaluation of the results was performed according to the International Organization for Standardization (ISO; ISO 3534 standard) and 5725-2 Guideline.

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[1] Authors to whom correspondence should be addressed; e-mail: f.bordet@afssa.fr and d.inthavong@afssa.fr.

Because of their persistence in the environment and their chronic toxicity in humans and animals (1–4), organochlorine pesticide (OCP) treatment of foods has been limited in industrialized countries in the last 2 decades. Lindane was the last to be banned from agricultural practice in France in 1998. Nevertheless, organochlorine residues are still found at low levels in milk, animal fat, and human milk everywhere in the world (5–9). The monitoring of OCP residue occurrence in food is a particular concern for officials in charge of human health protection (10, 11). European directives regulating pesticide residue levels in food incited European members in the monitoring of OCPs and pyrethroid pesticides (PPs; 12). Therefore, the establishment of maximum residue limits (MRLs) in foods, set by European Communities (13), urged the development of methods suitable for inspection programs.

Improvements have been recently noticed in the development of analytical methods (14): a low cost and rapid cleanup step using solid-phase extraction (SPE; 15–17), liquid–liquid partitioning (18), and low-temperature precipitation (19). Chromatography on Florisil® is usually performed as a cleanup step. In Europe, analytical standard NF-EN-1528 (20) is the reference tool for determination of OCPs and polychlorobiphenyls (PCBs) in animal products. This standard is a compilation of several methods that, for some, are not the most recent. Furthermore, PPs are not included in this standard. The recommended liquid–liquid partition and cleanup process are time- and solvent-consuming. SPE has been used for the purification of pesticide residues for several years (21), and many kinds of solid sorbents are now commercially available and can be used as the cleanup step adapted to specific groups of pesticides.

Proficiency tests have already been conducted on pesticides (22, 23), and national and international programs have been established to minimize the analytical variability. In

France, proficiency tests are undertaken regularly as part of monitoring programs to maintain data reliability. Analytical data variability, especially accuracy, is critical when estimations are conducted at a low level, between 10 and 50 ng/g. Because of limited information on method or laboratory performance, it is difficult to assess the reliability of data in France. Variability in organochlorine compounds analysis was studied previously by Miskiewicz and Gibbs (24), who emphasized "the difficulties inherent in analysis for low concentrations of organochlorine residues and the inherent variability in such analysis."

The present study reports the results of an interlaboratory study of OCP, PP, and PCB determination in 4 food matrixes undertaken with this method, which enlarges the scope of the EN standard to PPs; includes the use of SPE technique for cleanup; and measures the bias for OCPs, PPs, and PCBs in fat, milk, fish, and eggs at current levels.

Interlaboratory Study

Preparation of Test Materials

Milk samples.—Five liters of raw milk were bought in a supermarket. An aliquot of 1 L was reserved for blank tests. The batch was spiked with 360 μg of 2 standard mixtures of OCPs and PCB congeners to obtain a spiked level of ca 40 ng/g milk fat for each residue. PPs were not tested because they are not included in the monitoring program for milk. The milk batch was stirred for 12 h at 20°C, stabilized with 4 g sodium azide at 15% in water, and subdivided into 60 mL glass bottles. All samples were stored at 4°C for 2 weeks maximum before analysis.

Fish samples.—One salmon and one mackerel were used to provide the required quantity of fish muscle and to minimize the matrix effects. Filets (975 g total) were cut into pieces (2 cc), crushed, and thoroughly mixed with 1 kg anhydrous sodium sulfate to obtain a dry powder (25). A 350 g portion was kept as blank matrix. The fish batch was subdivided into 20 g samples in glass bottles, spiked separately with 1 mL OCP solution at 120 ng/mL and 0.5 mL PCB solution at 170 ng/mL, and then stored at 4°C for 2 months maximum before analysis. The concentrations used in the spiking procedure were chosen on the basis of expected residual content of ca 40 ng/g fat for pesticides and 60 ng/g fat for PCBs.

Beef fat samples.—About 3 kg beef fat tissue was cut into pieces (2 cc), and each batch of ca 500 g was melted at 50°C. Liquid fat was filtered on a paper filter and 60 g fat was spiked by using 3 standard solutions containing 1.5 μg OCP, 6 μg PP, and 2.4 μg PCB. The rest was kept as blank. The fat batch was stirred at 30°C for 12 h, and 2.5 g portions were placed in glass bottles, and preserved at 4°C.

Eggs samples.—Fifteen eggs, equivalent to 790 g without shell, were mixed and spiked with OCPs and PCBs to a content of ca 10 ng/g fat (OCPs) and 15 ng/g fat (PCBs). The mixtures were then stirred for 12 h before being subdivided into 50 g samples and were placed in glass bottles. They were kept at 4°C for 1 month maximum before analysis.

Table 1. Retention times, LOQ, MRL, and spike level for the 34 compounds

Compound	Retention time, min	LOQ[a], ng/g	MRL[b] in fat, ng/g	Spike level in fat, ng/g
α-HCH	15.200	2.7	200	25
β-HCH	15.613	2.3	100	25
HCB	16.805	2.8	200	25
γ-HCH	16.201	2.7	1000	25
Heptachlor	19.887	2.7	200[c]	25
Aldrin	20.045	3.0	200[c]	25
Heptachlor-epoxide	21.246	3.1	200[c]	25
Oxychlordane	21.408	3.0	50[c]	25
γ-Chlordane	22.116	2.9	50[c]	25
op'-DDE	22.350	2.5	1000[c]	25
α-Endosulfan	22.698	3.0	100[c]	25
α-Chlordane	23.833	2.9	50[c]	25
pp'-DDE	23.750	2.5	1000[c]	25
Dieldrin	24.846	2.8	200[c]	25
op'-TDE	24.981	2.8	1000[c]	25
Endrin	24.760	3.6	50	25
pp'-TDE	25.642	2.3	1000[c]	25
op'-DDT	26.173	2.4	1000[c]	25
pp'-DDT	28.316	1.9	1000[c]	25
op'-Dicofol	31.708	5.4	50[c]	25
Dicofol	32.495	2.2	50[c]	25
PCB 28	18.257	2.6	—[d]	40
PCB 52	19.331	2.5	—	40
PCB 101	22.527	2.4	—	40
PCB 118	25.494	2.2	—	40
PCB 153	27.037	2.6	—	40
PCB 138	28.691	2.4	—	40
PCB 180	34.000	2.4	—	40
λ-Cyalothrin	37.099	1.9	500	100
Permethrin	40.208	10.3	500	100
Cyfluthrin:[e]	42.044	11.7	50	100
1, 2, 3, 4	42.910			
Cypermethrin:[e]	43.160	7.8	200	100
1, 2, 3, 4	44.008			
Fenvalerate:[f]	46.451	7.2	500	100
1, 2	47.243			
Deltamethrin	49.099	1.9	50	100

[a] Validation results obtained with the standard NF V-03-110.
[b] Refs. 12 and 13.
[c] Sums of aldrin and dieldrin, chlordanes, DDTs, heptachlors, endosulfan, and dicofols.
[d] — = No MRL for PCBs.
[e] Four peaks. Maximum and minimum retention times. MRLs equal the sum.
[f] Two peaks. MRLs equal the sum.

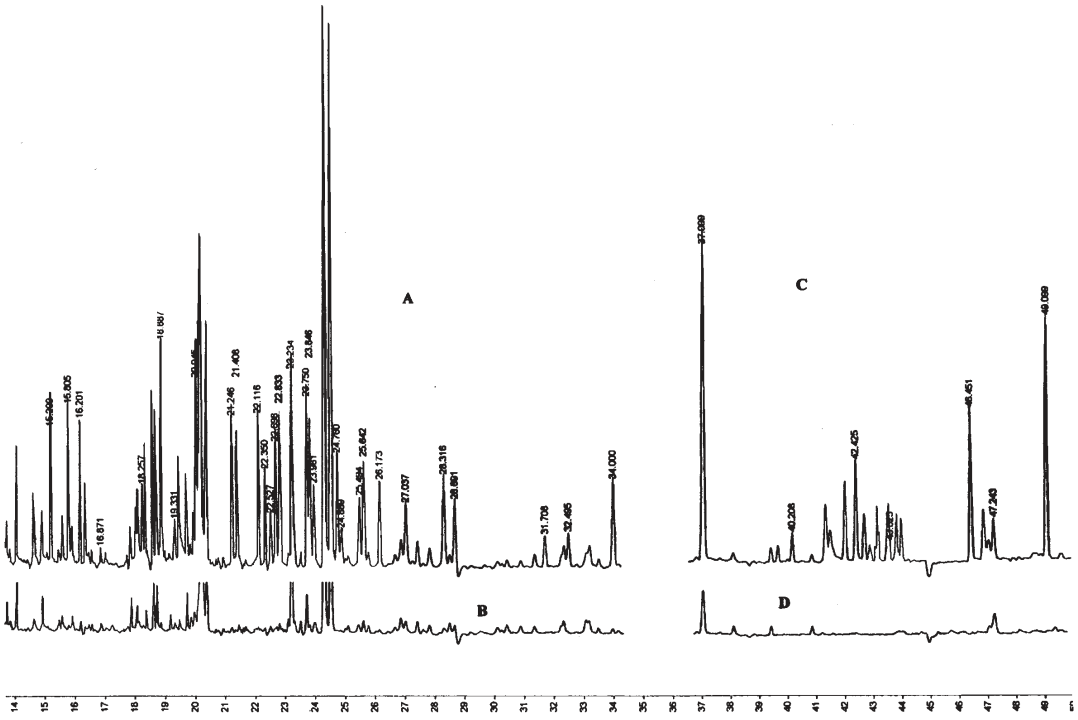

Figure 1. Chromatograms of blank and spiked samples. (A) Spiked fish sample at 40 ng/g for OCPs and 60 ng/g for PCBs. Retention times (min), *see* Table 1. Internal standard, transnonachlor. RT: 23.234 min. (B) Blank fish sample. (C) Spiked fat sample at 100 ng/g for PPs; part of chromatogram with PPs. (D) Blank fat sample.

Chromatographic parameters.—Retention times and limits of quantitation (LOQ; Table 1) were obtained with the method presented below. Figure 1 shows a chromatogram of blank and spiked samples.

Homogeneity test.—For all spiked test materials, 10 samples were randomly taken and analyzed in duplicate with the method described below. A "one-factor variance test" was performed. Results for fish samples are given in Table 2. Fish was the most problematic matrix because it is not a liquid matrix; however, the *F*-test proved the good homogeneity of the fish samples.

Organization of the Interlaboratory Study

Seventeen laboratories from 17 different French departments involved in pesticide monitoring programs participated in the interlaboratory study. These 17 laboratories received the official agreement by the French Ministry of Agriculture through national proficiency testing. They were trained for analysis of pesticides in fat for several years. For the interlaboratory trial, each participant received the following: (*1*) a set of 3 randomly coded samples of 60 mL liquid milk; (*2*) a set of 4 randomly coded samples of 20 g mixed fish; (*3*) one randomly coded sample of 50 g mixed eggs; (*4*) one randomly coded sample of 2.5 g fat; (*5*) one vial of 2 mL calibrated solution containing 35 substances with known concentration (between 10 and 40 pg/μL); (*6*) a copy of the method of analysis; (*7*) additional recommendations; (*8*) 4 forms for reporting results.

Participants were asked to calibrate their own chromatographic system and standard solution with the calibrated solution. To quantify each analysis (3 for each product: fat, milk, fish, and eggs) they were to use their own standard solution. The use of at least one internal standard (1S) was recommended for calculation. It was also specified not to freeze the milk sample and to totally extract the fish samples. Participants were instructed to use exactly the protocol provided, i.e., extraction, purification, and gas chromatography (GC) determination. Results were to be expressed in ng/g fat for each product without any correction factor. Other technical aspects, such as solvent quality, SPE procedure, and chromatographic material, were not under control.

METHOD

Principle

Pesticide extraction procedures were based on standard NF-EN-1528 Part 2, "Cold Centrifugation Extraction." For raw milk, AOAC extraction was performed. NF-EN-1528 standard does not mention cold centrifugation extraction for eggs; nevertheless, this extraction procedure was applied for eggs in the required protocol. The same cleanup protocol with SPE based on ready-to-use cartridges was set up for each product. Two types of silica cartridges were used successively: C_{18} and Florisil. GC was used to determine residue levels.

Table 2. Homogeneity test for fish samples. Variance analysis with one factor (*F* critic = 4.4939 at the risk of 5%)

Compound	*F* observed
α-HCH	0.0
β-HCH	0.0427
HCB	0.2557
γ-HCH	0.2674
Heptachlor	0.2195
Aldrin	0.0
Heptachlor-epoxide	1.4925
Oxychlordane	0.3333
γ-Chlordane	0.1369
op′-DDE	0.1963
α-Endosulfan	0.0
α-Chlordane	0.1856
Dieldrin	0.0
op′-TDE	1.7276
Endrin	0.0645
pp′-TDE	0.1461
op′-DDT	0.9110
pp′-DDT	0.0872
PCB 28	0.0
PCB 101	0.0007
PCB 180	0.0070

Apparatus

(**a**) *Refrigerated centrifuge*.—Able to rotate at 3000 rpm at –15°C.

(**b**) *Rotary evaporator*.—With vacuum device and cooler.

(**c**) *SPE automate*.—(Optional) or SPE vacuum manifold and vacuum pump.

(**d**) *Gas chromatograph system*.—With injection device and electron capture detector.

(**e**) *Capillary column*.—Nonpolar dimethyl-polysiloxane (100%) phase, thickness 0.25 μm, 50 m × 0.32 mm id.

(**f**) *Pre-column*.—1.5 m × 0.32 mm id.

(**g**) *Volumetric pipets*

Reagents

(**a**) *Pesticide standard solution in hexane*.—Hexachlorobenzene (HCB); endrin; α-HCH; *pp′*-TDE; β-HCH; *op′*-DDT; γ-HCH; *pp′*-DDT; heptachlor; *op′*-dicofol; aldrin; dicofol; heptachlor-epoxide; oxychlordane; γ-chlordane; *op′*-DDE; α-endosulfan; α-chlordane; *pp′*-DDE; dieldrin; *op′*-TDE; PCB congeners 28, 52, 101, 118, 153, 138, and 180, all at 10 ng/mL; deltamethrin, all at 8 ng/mL; permethrin, cypermethrin, fenvalerate, cyfluthrin at 40 ng/mL; and λ-cyalothrin at 10 ng/mL.

(**b**) *IS solution in isooctane*.—Transnonachlor at 10 μg/mL.

(**c**) *Acetone*

(**d**) *Diethyl ether*

(**e**) *Petroleum ether*

(**f**) *N-hexane*

(**g**) *Acetonitrile*

(**h**) *Methanol*

(**i**) *Methylene chloride*

(**j**) *Dodecane*.—Used as a keeper.

(**k**) *Sodium sulfate*.—Anhydrous.

(**l**) *Filter paper*.—Whatman No. 110 or equivalent.

(**m**) *Nonpolar SPE cartridge C_{18}*.—1 g, 6 mL, particle size 40 μm, pore size 60 Å (Varian or equivalent).

(**n**) *Polar SPE cartridge Florisil*.—1 g, 6 mL, particle size 200 μm [Varian (Sunnyvale, CA) or equivalent].

(**o**) *Mobile phase*.—Helium 99.99% purity, filtered for oxygen and water.

General Fat Extraction

(**a**) *For milk*.—AOAC extraction (NF-EN-1528) is performed. Shake 50 mL milk with 5 mL methanol and 0.5 g sodium oxalate for 1 min in 250 mL separating funnel. Add 25 mL diethyl ether and shake again for 1 min. Repeat with 25 mL petroleum ether. After separating the phases (centrifuging for 5 min at 1500 rpm may be required), transfer the organic phase into another separating funnel and extract the aqueous phase twice with 50 mL portions mixture (1 + 1, v/v) diethyl ether and petroleum ether. Wash combined solvent extracts over sodium sulfate anhydrous layer and evaporate by using rotary evaporator at ca 35°C.

(**b**) *For beef fat*.—Melt sample at ca 30°C. Filter melted fat on filter paper at ca 40°C. The organizing laboratory prepared this sample and collaborators received the extracted fat.

(**c**) *For fish*.—Add 50 mL *n*-hexane to 20 g fish. Mix, and centrifuge for 5 min at 1500 rpm. Decant upper phase and repeat extraction with 50 mL *n*-hexane. Keep the 2 extracts together. Evaporate solvent at ca 35°C to 1 mL, and finish evaporation with gentle stream of nitrogen.

(**d**) *For eggs*.—Use extraction column. In a beaker, carefully mix 15 g sand, 15 g sodium sulfate anhydrous, and then 10 g sample. Stopper a glass column with cotton-wool swab, add 2 cm sodium sulfate anhydrous and pour in the above preparation. Elute with 170 mL *n*-hexane and acetone (2 + 1, v/v). Evaporate solvent at ca 35°C to 1 mL, and finish evaporation with gentle stream of nitrogen.

Pesticide and PCB Extraction

For each product, pesticides and PCBs are extracted from fat by cryogenic extraction. Weigh 0.5 g fat extract in centrifuge tube, add 3 mL acetonitrile–methylene chloride (75 + 25, v/v), and mix vigorously. Centrifuge 20 min at 3000 rpm and ca –15°C. Keep upper-layer supernatant, and then slowly heat bottom to melt fat and repeat extraction with 3 mL of the same solvent mixture. Evaporate organic phase at ca 35°C under nitrogen to ca 2 mL (solution A).

Table 3. Interlaboratory study results for OCPs, PPs, and PCBs in animal fat[a]

Compound	Assigned value, ng/g	Mean recovery, %	No. of labs[b]	s_r	RSD_r, %	s_R	RSD_R, %	HORRAT
α-HCH	25	100	16 (0)	2.7	11	8.7	34	1.2
β-HCH	26	104	15 (0)	2.4	9	10	39	1.3
HCB	19	76	15 (1)	1.9	10	7.4	39	1.3
γ-HCH	27	108	16 (1)	2.2	8	8.3	33	1.2
Heptachlor	25	100	16 (1)	2.7	11	8.6	34	1.2
Aldrin	21	84	16 (0)	2.1	10	8.4	40	1.3
Heptachlor-epoxide	29	116	14 (0)	3.1	10	11.4	40	1.5
Oxychlordane	28	112	14 (0)	2.5	9	11.3	41	1.5
γ-Chlordane	26	104	15 (0)	2.9	11	9.0	35	1.2
op′-DDE	24	96	14 (0)	2.4	10	8.9	36	1.3
α-Endosulfan	28	112	16 (0)	2.9	10	9.6	34	1.2
α-Chlordane	27	108	16 (0)	2.8	10	9.5	36	1.3
pp′-DDE	28	112	16 (1)	2.4	8	10.4	38	1.4
Dieldrin	30	120	16 (0)	2.8	9	9.0	30	1.1
op′-TDE	32	128	15 (0)	2.8	8	10.1	32	1.2
Endrin	30	120	16 (0)	2.5	8	12.7	42	1.5
pp′-TDE	29	116	15 (1)	3.1	10	10.6	36	1.3
op′-DDT	29	116	15 (0)	3.4	12	9.2	32	1.2
pp′-DDT	29	116	15 (0)	2.5	8	9.6	33	1.2
op′-Dicofol	38	152	9 (1)	2.9	7	17	47	1.8
Dicofol	21	84	10 (0)	2.6	12	6.2	29	1.0
PCB 28	34	136	16 (0)	3.4	10	10.9	32	1.2
PCB 52	38	152	15 (0)	3.2	8	15.2	40	1.5
PCB 101	38	152	16 (0)	2.6	7	18.8	49	1.8
PCB 118	37	148	15 (0)	3.5	9	18.8	51	1.9
PCB 153	32	128	16 (0)	2.7	8	11.2	35	1.2
PCB 138	31	124	16 (1)	2.4	8	11.1	37	1.3
PCB 180	30	120	15 (1)	2.7	9	11	38	1.4
λ-Cyalothrin	14	14	15 (3)	1.9	13	8.8	62	2.0
Permethrin	127	127	14 (1)	12	10	49	39	1.7
Cyfluthrin	85	85	14 (3)	10	12	36	43	1.8
Cypermethrin	118	118	15 (2)	8.6	7	45	38	1.7
Fenvalerate	118	118	15 (2)	11	10	47	40	1.8
Deltamethrin	32	32	12 (1)	3.5	11	12	39	1.4
Average[c]	38			3.6		14.7		

[a] Spiked values: OCPs, 25 ng/g; PCBs, 40 ng/g; PPs, 100 ng/g.

[b] Total number of laboratories; 16 laboratories participated for fat. The number of laboratories removed as outliers is indicated in parentheses. Exclusion was based on internal variance test.

[c] Pyrethroid excluded.

Cleanup

C_{18} SPE.—Process cartridge with 5 mL petroleum ether, 5 mL acetone, and 5 mL methanol twice, eluted to meniscus. Solution A (2 mL) is loaded into cartridge and eluted to meniscus (keep 3 min in contact). Wash solution A container with 10 mL acetonitrile, load it into cartridge, and elute (1 drop/3 s). Elutant is evaporated at about 35°C with 100 µL dodecane; then dilute in *n*-hexane (solution B).

Florisil SPE.—Process cartridge with 10 mL *n*-hexane eluted to meniscus. Load solution B (3 min contact) and elute with 10 mL petroleum ether–diethyl ether (98 + 2, v/v; 1 drop/s); and 12 mL petroleum ether–diethyl ether (85 + 15, v/v; 1 drop/3 s). Melt the 2 fractions and evaporate together with 100 µL dodecane. Dissolve final extract in appropriate volume of *n*-hexane for GC analysis (solution C).

Gas Chromatographic Determination

(**a**) *Internal control procedure.*—Before analysis, participants must determine linearity and determination limit of their own GC system. They are advised to use a certified reference material, which is a product at least equivalent to one of the 4 products included in the present interlaboratory study.

(**b**) *Operating conditions.*—When column (e.g., CPSil 5) and mobile phase helium were used, the following settings were appropriate: helium stream pressure 23 psi; initial oven temperature 100°C, holding time 2 min, rate 7°C/min; temperature 220°C, holding time 10 min, rate 3°C/min; final temper-

Table 4. Interlaboratory study results for OCPs and PCBs in milk[a]

Compound	Assigned value, ng/g	Mean recovery, %	No. of labs[b]	s_r	RSD_r, %	s_R	RSD_R, %	HORRAT
α-HCH	39	98	15 (3)	3.4	3.4	17.6	45	1.7
β-HCH	33	83	14 (2)	3.1	3.1	10.7	33	1.2
HCB	27	68	14 (0)	5.2	5.2	12.7	46	1.6
γ-HCH	40	100	15 (2)	3.4	3.4	17.4	43	1.7
Heptachlor	36	90	15 (2)	4.1	4.1	12.5	35	1.3
Aldrin	31	78	15 (1)	2.7	2.7	11.8	38	1.4
Heptachlor-epoxide	45	113	15 (1)	3.9	3.9	18.6	42	1.6
Oxychlordane	38	95	13 (0)	3.4	4.9	19.9	50	1.9
γ-Chlordane	37	93	15 (1)	5.1	5.1	14.8	39	1.5
op′-DDE	34	85	14 (1)	5.1	5.1	14.1	41	1.5
α-Endosulfan	38	95	15 (0)	5.2	5.2	16.5	43	1.7
α-Chlordane	39	98	15 (0)	1.5	1.5	16.6	43	1.7
pp′-DDE	36	90	15 (0)	5.7	5.7	15.8	44	1.7
Dieldrin	41	103	15 (0)	5.7	5.7	16.4	40	1.5
op′-TDE	41	103	14 (0)	4.9	4.9	16.8	39	1.5
Endrin	42	105	15 (0)	6.0	6	16.0	39	1.5
pp′-TDE	41	103	15 (1)	5.2	5.2	19.4	43	1.7
op′-DDT	36	90	13 (2)	4.1	4.1	11.9	33	1.3
pp′-DDT	39	98	14 (1)	5.5	5.5	14.6	42	1.6
op′-Dicofol	45	113	7 (1)	9.1	9.1	30.4	67	2.6
Dicofol	45	113	9 (0)	5.5	5.5	11.5	67	2.7
PCB 28	45	113	15 (0)	6.8	6.8	20.8	46	1.8
PCB 52	38	95	14 (0)	6.3	6.3	17.2	46	1.8
PCB 101	32	80	15 (1)	3.1	5.6	11.2	35	1.3
PCB 153	30	75	15 (1)	4.3	4.3	11.0	37	1.4
PCB 138	26	65	15 (0)	3.7	3.7	12.8	42	1.6
PCB 180	26	65	14 (1)	6.2	6.2	11.1	43	1.5
Average	37	98		4.7		15.6		

[a] Spiked value = 40 ng/g.

[b] Total number of laboratories; 15 laboratories participated for milk. The number of laboratories removed as outliers is indicated in parentheses. Exclusion was based on internal variance test. PCB 118 is not present. PPs are not included. For dicofols *see* text.

ature 285°C, injector initial temperature 50°C, rate 150°C/min; final temperature 250°C; holding time 52 min; detector temperature 320°C; nitrogen make-up detector at 25 mL/min; injection volume 1 μL. Data sets were produced by GC–electron capture detection (ECD).

(**c**) *Evaluation.*—Determine, from calibration chromatogram, masses in ng/g fat of solution injected onto GC column. Calculate mass (M) of unknown substance (US) in ng/g using the equation or programming computer:

$$M = \left[\frac{A}{B}\right] \times \left[\frac{D}{C}\right] \times \left[\frac{E}{F}\right] \times G \times H$$

where A = US peak height in sample, B = IS peak height in sample, C = US peak height in working solution, D = IS peak height in working solution, E = concentration of US in pesticide standard solution, F = mass of sample, G = final volume of extract, and H = dilution factor.

Results and Discussion

Interlaboratory Trial Results Study

Data were received from 16 participants for fat with 34 substances and 3 repetitions ($16 \times 34 \times 3 = 1632$ data), 15 participants for milk ($15 \times 27 \times 3 = 1215$ data), 14 participants for fish ($14 \times 26 \times 3 = 1090$ data), and 12 participants for eggs ($12 \times 26 \times 3 = 936$ data), with 26 or 27 substances and 3 repetitions for the last 3. Not all data sets were completed, either because of a deficiency in results for some compounds or lack of confirmation on a second GC column. The results were screened to remove invalid data. "Invalid data are results from laboratories that did not follow instructions, did not achieve

Table 5. Interlaboratory study results for OCPs and PCBs in fish[a]

Compound	Assigned value, ng/g	Mean recovery, %	No. of labs[b]	s_r	RSD_r, %	s_R	RSD_R, %	HORRAT
α-HCH	43	108	13 (1)	3.4	7.9	16.0	36	1.5
β-HCH	44	110	13 (0)	3.4	7.7	13.6	31	1.3
HCB	31	78	11 (2)	3.7	12.3	13.3	43	1.6
γ-HCH	41	103	12 (2)	2.1	4.5	18.0	44	1.7
Heptachlor	37	93	11 (2)	3.6	9.2	18.6	50	1.9
Aldrin	32	80	14 (0)	4.8	14.1	14.5	46	1.7
Heptachlor-epoxide	43	108	11 (2)	2.6	6.0	19.9	47	1.8
Oxychlordane	39	98	11 (0)	4.1	7.4	18.9	46	1.7
γ-Chlordane	42	105	14 (1)	3.9	7.6	21.0	51	1.8
op′-DDE	38	95	13(3)	3.9	10.2	19.6	51	1.9
α-Endosulfan	47	118	14 (1)	5.0	9.1	21.5	45	1.8
α-Chlordane	44	110	14 (2)	4.3	9.7	23.4	53	1.9
pp′-DDE	66	165	14 (1)	5.9	8.4	27.6	42	1.7
Dieldrin	54	135	12 (1)	6.3	11.6	25.5	47	1.9
op′-TDE	49	123	13 (1)	6.9	12	23.5	50	2
Endrin	57	143	14 (1)	7.1	12	27	51	2
pp′-TDE	60	150	14 (1)	7.1	11	24	51	2
op′-DDT	42	105	13 (1)	3.6	8.4	15.4	38	1.5
pp′-DDT	52	130	13 (2)	5.9	12	28	54	2.1
PCB 28	54	90	14 (1)	3.6	6.6	22.8	42	1.7
PCB 52	48	80	13 (0)	5.2	10.8	20.1	42	1.7
PCB 101	59	98	14 (1)	7.2	12.2	30.8	52	2.1
PCB 118	23	38	13 (0)	3.7	16	13.6	57	2
PCB 153	66	110	14 (1)	7.0	10	31.7	48	2
PCB 138	66	110	14 (2)	4.4	6.6	27.2	41	1.7
PCB 180	40	67	14 (1)	3.9	9.7	18	44	1.7
Average	47			4.7		21.3		

[a] Spiked values = OCPs, 40 ng/g; PCBs, 60 ng/g.

[b] Total number of laboratories; 14 laboratories participated for fish. The number of laboratories removed as outliers is indicated in parentheses. Exclusion was based on internal variance test. PPs are not included.

the expected separations or traced to a specific cause" (26). Some laboratories could not reliably detect some pesticides.

Participants were advised to use at least 2 columns with stationary phases of different polarity to exclude false-positive data caused by co-eluted compounds. Automatic injection was recommended. Nevertheless, several results did not appear to be reliable. For example, 7 laboratories reported a positive result for PCB 118 in a milk sample, but PCB 118 was not added in this matrix. A milk sample chromatogram was then requested from each participant. A detailed examination of these chromatograms shows a peak at the retention time of PCB 118. This demonstrates the absolute requirement to inject in a double GC column or to use a mass detector for a clear identification of the presence of pesticides. Doubtful results were excluded based on nonrespect of recommendations or unidentified obvious mistakes.

The method was previously validated in-house by using the French standard NF V-03-110 (unpublished results) and, therefore, linearity, limit of detection (LOD), and LOQ (Table 1) were calculated for each pesticide. LODs were <2 ng for all OCPs and PCBs and <3 ng for PPs. During the in-house validation, linearity was tested from the LOD value to 25 ng for OCPs and PCBs and from the LOD value to 100 ng for PPs. Recommendation was made to the participants to determine their own linearity range and to dilute extracts if necessary.

Interpretation of Results

Precision estimates were obtained according to the International Union of Pure and Applied Chemistry (IUPAC) International Harmonized Protocol (27). Mean levels, precision parameters, and HORRAT values are given in Tables 3–6. For

Table 6. Interlaboratory study results for OCPs and PCBs in eggs[a]

Compound	Assigned value, ng/g	Mean recovery, %	No. of labs[b]	s_r	RSD_r, %	s_R	RSD_R, %	HORRAT
α-HCH	5.8	58	12 (2)	0.7	12	2.0	34	1
β-HCH	7	70	10 (2)	1.2	17	2.2	32	0.9
HCB	4	40	9 (2)	0.6	15	1.9	47	1.3
γ-HCH	8.7	87	12 (2)	0.5	6	3.7	32	1.3
Heptachlor	6.8	68	11 (1)	0.7	10	3.3	49	1.5
Aldrin	6.0	60	11 (1)	1.0	16	4.6	77	2.2
Heptachlor-epoxide	8	80	11 (1)	0.8	10	3.8	47	1.4
Oxychlordane	8.5	85	9 (0)	0.9	10	3.6	42	1.3
γ-Chlordane	7.7	77	12 (2)	0.9	11	3.2	42	1.3
op′-DDE	7.2	72	11 (0)	1.1	15	4.4	61	1.8
α-Endosulfan	8.6	86	12 (1)	1.0	11	2.5	29	0.9
α-Chlordane	7.8	78	12 (1)	0.8	10	4.6	58	1.7
pp′-DDE	7.6	76	12 (2)	0.6	8	3.0	41	1.2
Dieldrin	10	100	12 (1)	0.9	9	3.8	38	1.2
op′-TDE	11.5	115	10 (0)	2.0	17	5.9	52	1.7
Endrin	9	90	12 (2)	0.6	6	3.9	43	1.3
pp′-TDE	7.6	76	9 (0)	0.7	9	2.5	33	1.0
op′-DDT	27	270	10 (0)	0.9	3	3.0	41	1.2
pp′-DDT	8.2	82	11 (1)	3.2	39	4.7	57	1.7
PCB 28	13	87	11 (2)	1.0	8	7.7	59	1.9
PCB 52	16	107	10 (0)	1.9	11	7.3	44	1.5
PCB 101	10.8	72	11 (1)	1.0	9	4.4	41	1.3
PCB 118	12.8	85	10 (0)	1.5	12	4.6	36	1.2
PCB 153	10.3	69	11 (2)	1.0	10	4.2	41	1.3
PCB 138	10.5	70	11 (1)	1.2	11	3.8	37	1.2
PCB 180	9.3	62	11 (1)	1.1	12	4.7	50	1.6
Average	9.6			1.1		3.9		

[a] Spiked values = OCPs, 10 ng/g; PCBs, 15 ng/g.

[b] Total number of laboratories; 12 laboratories participated for eggs. The number of laboratories removed as outliers is indicated in parentheses. Exclusion was based on internal variance test. For aldrin *see* text.

each sample, some participants did not give results for each compound, generally because of a chromatographic resolution problem. Participants were better trained for fat and milk than for fish and eggs: 2 and 4 participants did not give any result for fish and eggs, respectively; 17 results (on 1632 data) are missing for fat (1%), 8 for milk (0.6%), 16 for fish (1.3%), and 22 for eggs (2.1%). Participants had a better control for fat and milk, which is reflected in their RSD_r. Based on HORRAT values, results were good for eggs, but *pp′*-DDT RSD_r (39%), *op′*-TDE (17%), and β-HCH (17%), and other pesticides with RSD_r of 15 or 16%, reflect that the method was not perfectly controlled by participants for this matrix.

Precision Characteristics of Method

Precision characteristics of the method were assessed by HORRAT values, which compare the RSD_R obtained for a particular matrix with that statistically predicted on the basis of interlaboratory trial studies taken from published literature. All HORRAT values ≤2.0 in this study indicate acceptable precision. Some exceptions are for *pp′*-DDT and PCB 101 in fish (2.1) and aldrin in eggs (2.2). The case of dicofols in milk (2.6 and 2.7) is discussed below.

Mean levels, precision parameters, and HORRAT values are given in Tables 3–6. The statistical evaluation was performed on uncorrected data according to the French standard NF V-03-110. Data (4873 total) were excluded on the average only for internal variance with a Cochran test. We chose this strict option to give a value of a full validation method to this exercise. Thus, outliers were excluded with the Cochran test only for internal variance (*see* column "No. of labs" in Tables 3–6).

HORRAT values from each matrix are compared in Figure 2. The lowest HORRAT values were observed for fat matrix (average = 1.4) and eggs (average = 1.38). Nevertheless, this parameter is scattered between 0.9 and 2.2 in eggs. HORRAT values around 1.5 are more targeted for milk. In contrast, this precision value is higher in fish, with HORRAT values >1.5 (average = 1.79), reaching and exceeding 2 for some pesticides. Global HORRAT values are acceptable for low levels of pesticides in matrixes, much lower than MRLs adopted in Europe. Intra- and interlaboratory variability, reflected in RSD_r and RSD_R values, are acceptable. Differences between added amounts in spiked samples and consensual means are low (*see* "Mean recovery, %", Tables 3–6). The choice was to consider the overall mean as the assigned value and not the added amount.

It is important that poor results for λ-cyalothrin and deltamethrin are not a problem. These poor recoveries could be explained by particular heat instability of these compounds inside chromatographic apparatus. These results do not ban performance estimation.

The general performances of the method are discussed. HORRAT values stand between 1 and 2, considering that 2 is still acceptable. The most critical step of the method is the cleanup step. After extraction and cryogenic extraction, the extract is loaded into a C_{18} cartridge to eliminate fat and into a second Florisil cartridge to eliminate more polar interferences. These 2 cleanup steps are standardized for the 4 matrixes, which are not similar. The fatty acids profile of triglycerides or free fatty acid is not the same in milk, animal fat, eggs, or fish. For example, light fatty acids are found more often in milk than in fat, specific fatty acid with long chains are present in fish, and eggs are loaded with cholesterol.

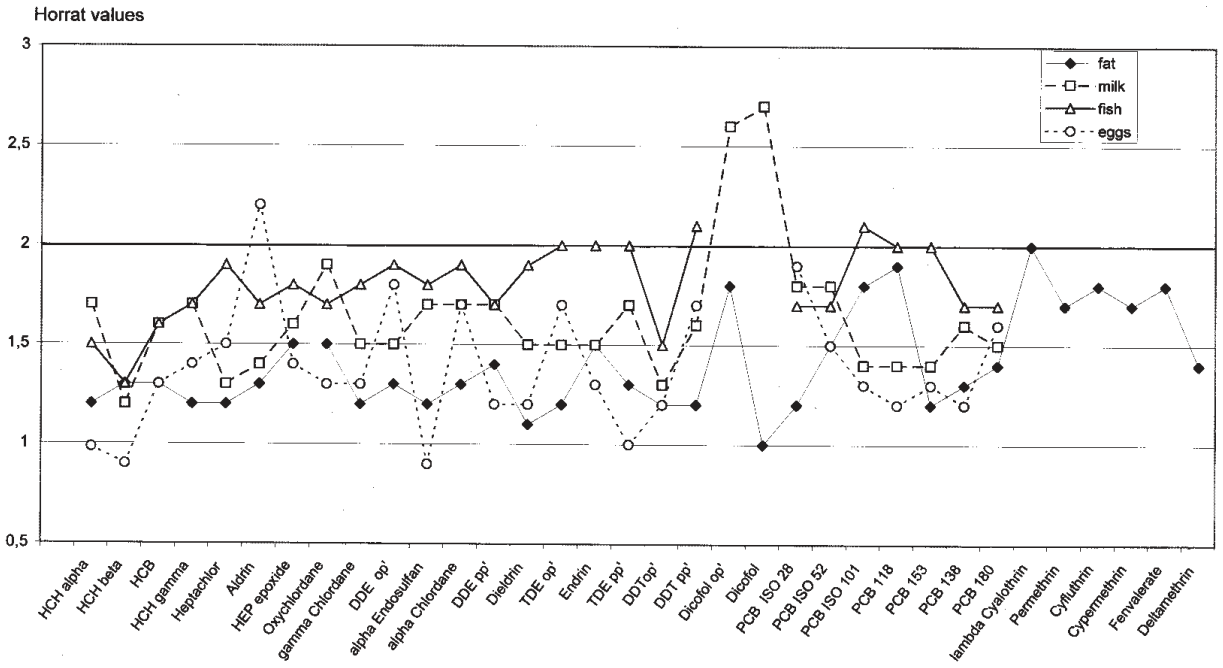

Figure 2. Comparison of HORRAT values achieved for each matrix.

C_{18} and Florisil cartridges do not work in the same polarity range environment and do not retain pesticides in the same way. Foreign matter, co-extracted previously, may or may not be adsorbed, depending on their own polarity. Co-extracted matter can interfere with chromatographic diagram. In developing an analytical method suitable for such different matrixes, we must expect some compromise in acceptable performance criteria. We could improve pesticide performances by eluting a larger volume in cartridges, but that procedure would probably increase interferences, which could be an obstacle for a reliable identification and quantification. Chromatographic arrangement is possible with the use of a double chromatographic column device or a mass spectrometer detector.

Fatty content in matrixes is 100% for animal fat, 4% for milk, 10% for eggs, and 11% for fish. Considering these levels of fat content and the regulation rules for pesticides in Europe, results on fat material are reported.

Fat

Fat is the matrix for which participants received the best training. Among 34 residues, Laboratory 15 was eliminated for 8 of them. Most HORRAT values were <1.5 (65%). Highest HORRAT values were found for PPs. The analytical problem for these pesticides is that one compound gives several chromatogram peaks due to the presence of isomers. Participants were to give the result as the sum of the different peaks, but the risk of error is greater than if only one peak is considered. Although RSD_R values were not higher for PPs than for other pesticides, PP spiked levels were higher; consequently, RSD_R values should be lower. A low RSD_R involved a low Horwitz value. HORRAT values in our case are high because low Horwitz values correspond with high RSD_R. For the same reason, PCBs depict high HORRAT values.

For the 21 OCPs, the spiked level was 25 ng/g, and average recoveries were between 76 and 128%, excluding *op′*-dicofol. With a result of 38 ng/g (recovery 152%), *op′*-dicofol corresponded to the highest HORRAT value (1.8). For milk, determination of dicofols was usually a problem for several laboratories because of injection at too high a temperature. Only 6 values out of more than 300 were excluded. Values of s_r of between 2 and 3 gave an RSD_r between 8 and 11. Obviously, the RSD_R values seemed high, but HORRAT values between 1.1 and 1.5 were acceptable.

Table 3 shows average values of 19 to 32 ng/g for OCPs. Corresponding reproducibility of the NF-EN-1528 standard would be 16 at 19 ng/g and 23 at 32 ng/g. The corresponding s_R would be 6.7 at 19 ng/g and 8.2 at 32 ng/g ($s_R = R/2.8$), and the corresponding RSD_R would be 35% at 19 ng/g and 25% at 32 ng/g ($RSD_R = s_R/M$). In Table 3, RSD_R values are from 42 to 30% for OCPs, which leads to a HORRAT value >1 but <2, which is acceptable.

Milk

Despite a complicated protocol, HORRAT values are <1.9 for all OCPs and PCBs in milk except for dicofol compounds. The problem with these 2 compounds is that they are metabolized inside an injector set at too high a temperature. Two isomers exist: *op′*-dicofol, or 2,2,2-trichloro-1(2-chlorophenyl)-1(4-chlorophenyl) ethanol, and *pp′*-dicofol or 2,2,2-trichloro-1,1bis(4-chlorophenyl)ethanol. These molecules are hydrolyzed by light or by temperatures >80°C into 2,4′-dichlorobenzophenone and 4-4′-dichlorobenzophenone, respectively. Several participants did not report results for *op′*-dicofol (7 of 15) and for *pp′*-dicofol (9 of 15). Since this exercise, notification was sent to each participant to adjust the chromatographic conditions and to use a low-temperature on-column injector. The variability of results caused a difficult interpretation; consequently, the present method could not be validated for these compounds in milk. Table 4 shows average values of 26 to 45 ng/g for OCPs and PCBs. The corresponding reproducibility of the NF-EN-1528 standard would be 20 at 26 ng/g and 29 at 45 ng/g (excluding dicofols). The corresponding values would be 7.1 and 10.3 for s_R and 27 and 23% for RSD_R. Table 4 shows RSD_R values of 50 to 33% for OCPs, which leads to HORRAT values close to 2.

Fish

By mixing and drying the fish sample with sodium sulfate anhydrous, we were able to propose a nondesiccated tissue, which gave the participants an opportunity to include the extraction step as a part of the study. This sample gives the highest RSD_r result, which underlines analytical difficulties. Incomplete extraction of fat would not be a major problem for this matrix because these contaminants are easy to extract from highly fatty tissues (11% fat content for fish). Average results close to spiked values at 40 ng/g for OCPs and 60 ng/g for PCBs indicate a good recovery rate. However,

Table 7. Percentage of outliers removed by Cochran test

Matrix	Total data reported	Data removed by Cochran test, %	No. of labs for which data were removed 3 or more times	No. of labs for which data were removed 1 or 2 times
Eggs	864	3.2	19, 21, 23	13, 17
Fish	1014	2.9	15, 21	11, 12, 19
Fat	1530	1.2	11, 15	17, 19, 25
Milk	1134	1.8	19, 21, 23	13, 14, 18, 24

co-extraction and chromatographic co-elution of interfering materials can affect final results (Table 5). Our sample preparation, attempting to preserve a fresh matrix, may not be the best for that extraction protocol. HORRAT values in fish matrix are between 1.7 and 2. At worst, HORRAT values of *pp′*-DDT and PCB 101 in fish (2.1) are over the limit of 2. RSD_R values for these compounds are high (54 and 52), which indicate too large a dispersion between individual means. Similar difficulties were found during laboratory-performance study on cod liver (23) and on spiked fish (24). During our own in-house validation, we also observed such problems.

Table 5 shows average values of 31 to 66 ng/g for OCPs. Corresponding reproducibility of the NF-EN-1528 standard would be 22 at 31 ng/g and 38 at 66 ng/g. Corresponding values would be 7.8 and 13.6 for s_R and 25 and 20.6% for RSD_R. Table 5 shows RSD_R values of 54 to 31% for OCPs, which lead to a HORRAT value close or equal to 2.

Eggs

The specific difficulty with this matrix is that residues are at very low levels, from 4 ng/g for HCB to 27 ng/g for *op′*-DDT (Table 6). Only 12 laboratories participated in this exercise. In fact, it was the first exercise with this matrix for all participants. No previous training had been done. Even if RSD_R values seem to be high for several pesticides (*op′*-DDE , α-chlordane, *op′*-TDE, *pp′*-DDT), HORRAT values remained below the acceptable limit of 2 and were among the lowest of the 4 matrixes (average = 1.38). The aldrin in eggs shows an individual mean for Laboratory 21 of 17.4, whereas the general mean was 6.0. By excluding this laboratory, the mean becomes 4.9. The RSD_r is not changed, but RSD_R becomes 54 and the HORRAT value becomes 1.5. We determined that excluding this laboratory on the mean would be justified, as only one laboratory increases the general mean of 20% and the RSD_R of 23 points. Moreover, Laboratory 21 was often excluded for internal variance by Cochran test (Table 7), which excludes an unacceptable mean value at the same time. Statistical tests do not always take into account a systematic bias of only one laboratory may affect the bias of the method.

Table 6 shows average values of 4 to 11.5 ng/g for OCPs (except *op′*-DDT = 27). The corresponding reproducibility of the NF-EN-1528 standard would be 5 at 4 ng/g and 11 at 11.5 /g. Corresponding values would be 1.8 and 3.9 for s_R, and 45 and 34% for RSD_R. Table 6 shows RSD_R of 77 to 29% for OCPs, which leads to a HORRAT value >1 but <2 except for aldrin (2.2).

Conclusions

This study objectively examined the within- and between-laboratory accuracy of the described method. This method partly involves the European standard NF-EN-1528, which enlarges the scope to include PPs. The technique was improved by the use of SPE as a cleanup step. Two SPE cartridges, C_{18} and Florisil, were used successively. This interlaboratory study was performed on 4 different matrixes—animal fat, milk, fish, and eggs—spiked at levels lower than regulatory levels. In fact, most pesticide contents found in food samples are far below the regulatory levels. Thus, the effective parameters of the method can be observed at the lower rather than the higher level.

The analytical performance of laboratories was irregular as a result of inherent difficulties in the determination of low concentrations of pesticide residues. It is well known that confidence interval limits increase under 1 mg/kg and more drastically under 0.1 mg/kg. The European standard proposes reproducibility statistics from 0.01 at 0.01 mg/kg, to 0.05 at 0.1 mg/kg. Log-log extrapolation is performed in between.

Considering Horwitz and HORRAT values, the global results of this study are within the limits for OCPs, PPs, and PCBs, considering that an acceptable HORRAT value is <2. In the 4 matrixes, HORRAT values were generally between 1 and 2. Thompson and Lowthian (28) reviewed the Horwitz equation for levels under 0.01 mg/kg. Considering their experimental results, they proposed another calculation, $s_R = C/3$, derived from the IUPAC detection limit, which gave a better estimation of s_R than did the Horwitz prediction. The obtained s_R results on fat matrix in this study agree with this equation.

Regarding fat, the mean result was equal to 29 (PPs excluded) and the average s_R was equal to 11. However, s_R results obtained from milk, eggs, and fish do not always agree with the Thompson equation. The assigned values and average s_R values equal, respectively, 37 and 15 for milk, 9.6 and 3.9 for eggs, and 47 and 21 for fish. These gaps between theoretical and real results are also observed if we consider the RSD_R from the NF-EN-1528 standard. For fat and eggs, s_R values are close to the limit, which lead to HORRAT values close but above 1. HORRAT values are more often close to the extreme limit of 2 in milk and fish, because these matrixes are more problematic. For these matrixes, the RSD_R generally lies above the NF-EN-1528 standard.

Nevertheless, the accuracy for these 4 matrixes still stands in conformity with HORRAT values. PP performances are in the same range as those for other pesticides. Other studies depict a better RSD_R (22), but they were performed on technical and formulated materials or at a higher level.

The aim of this exercise was to demonstrate that, despite low spiking levels, the method can be used with a sufficient reliability. The relatively high variability in the determination of organochlorine compounds considered in this study was expected (29). Several remarks are highlighted from the results of this study. First, the variability in accuracy between laboratories was significantly within the acceptable limit. Second, the use of 1 g SPE cartridges was a limit in terms of purification for complex food matrixes. Prior fat extraction resulted in foreign matter that was not always adsorbed on the cartridge and could interfere with the pesticides in the cartridge or in the chromatographic column.

This work provides a common method in very different matrixes for 21 OCPs, 6 PPs, and 7 PCBs. It deals with SPE cleanup and low solvent volume. Compared with the European standard, the new validated method has a larger applicability and is less time- and solvent-consuming.

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References

(1) Itawa, H., Tanabe, S., Sakal, N., & Tatsukawa, R. (1993) *Environ. Sci. Technol*. **27**, 1080–1098

(2) Ferrando, M.D., Alarcon, V., Fernandeez-Casalderrey, A., Gamon, M., & Andreu-Moliner, E. (1992) *Bull. Environ. Contam. Toxicol*. **48**, 747–755

(3) Muir, D.C.G., Norstrom, R.J., & Simon, M. (1988) *Environ. Sci. Technol*. **22**, 1071–1079

(4) Collins, T. (2000) *Nature* **406**, 17–19

(5) Bordet, F., Mallet, J., Maurice, L., Borrel, S., & Venant, A. (1993) *Bull. Environ. Contam. Toxicol*. **50**, 425–432

(6) Waliszewski, S.M., Aguirre, A.A., Infanzon, R.M., Benitez, A., & Rivera, J. (1999) *Bull. Environ. Contam. Toxicol*. **62**, 685–690

(7) Dewailly, E., Weber, J.P., Gingras, S., & Laliberté, C. (1991) *Bull. Environ. Contam. Toxicol*. **47**, 491–498

(8) Collins, G.B., Holmes, D.C., & Hoodless, R.A. (1982) *Hum. Toxicol*. **1**, 425–431

(9) Okonkwo, J.O., Kampira, L., & Chingakule, D.D. (1999) *Swaziland. Bull. Environ. Contam. Toxicol*. **63**, 243–247

(10) Araujo, A.C.P., Telles, D.D., Gorni, R., & Lima, L.L.A. (1999) *Bull Environ. Contam. Toxicol*. **62**, 671–676

(11) Herrera, A., Arino, A., Conchellon, P., Lazaro, R., Bayarri, S., Perez-Arquillué, C., Garrido, M.D., Jodral, M., & Pozo, R. (1996) *Bull. Environ. Contam. Toxicol*. **56**, 173–177

(12) Directive 96/23/CE (1996) *Off. J. Eur. Commun.* **125**, 10–32

(13) Directive 86/363/CE (1986) *Off. J. Eur. Commun*. **363**, 1–17

(14) Quintanilla-Lopez, J.E., Lebron-Aguilar, R., & Polo-Diez, L.M. (1992) *J. Chromatogr*. **591**, 303–311

(15) Schenck, F.J., & Donoghue, D.J. (2000) *J. Agric. Food Chem*. **48**, 6412–6415

(16) Schenck, F.J., & Wagner, R. (1995) *Food Addit. Contam*. **12**, 535–541

(17) Gillespie, A.M., Daly, S.L., Gilvidis, D.M., Schneider, F., & Walters, S.M. (1995) *J. AOAC Int*. **78**, 431–437

(18) Tan, G.H. (1992) *Analyst* **117**, 1129–1132

(19) Bordet, F., Inthavong, D., Mallet, J., & Maurice, L. (1996) *Analusis* **24**, 328–333

(20) *Official Method of Analysis* (1997) NF-EN-1528, Association Française de Normalisation, Paris, France

(21) Bennett, D.A., Chung, A.C., & Lee, S.M. (1997) *J. AOAC Int*. **80**, 1065–1077

(22) Vargyas, L.D., Walls, G.E., & Bramstedt, W.R. (2000) *J. AOAC Int*. **83**, 1047–1052

(23) de Boer, J., & Wells, D.E. (1996) *Mar. Pollut. Bull*. **32**, 654–666

(24) Miskiewicz, A.G., & Gibbs, P.J. (1992) *Arch. Environ. Contam. Toxicol*. **23**, 45–53

(25) Kingston, D.W., Henry, M.A., Aldrin, K.J., & Pryde, S.D. (1994) *J. Chromatogr. Sci.* **32**, 383–385

(26) Horwitz, W. (1993) *Food Addit. Contam*. **10**, 61–69

(27) Thompson, M., & Wood, R. (1993) *J. AOAC Int*. **76**, 926–940

(28) Thompson, M., & Lowthian, P.J. (1997) *J. AOAC Int*. **80**, 676–679

(29) Horwitz, W., Jackson, T., & Chirtel, S.J. (2001) *J. AOAC Int*. **84**, 919–935