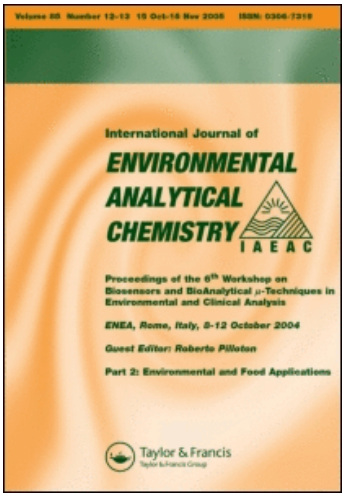

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In-house validation for analytical methods and quality control for risk evaluation of chlordecone in food

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Chlordecone was used until 1993 as a pesticide in the banana plantation of Martinique and Guadeloupe (French Antilles) against the root borer. This organochlorine pesticide was lipophilic, remnant, and toxic for human beings with both acute and chronic effects. Chlordecone was strongly absorbed and stored in soil and weakly decomposed in environment. Surveys conducted in 2001 revealed its presence in soil, rivers, and domestic food products. Local food (fruits and vegetables, cattle, poultry, and fish) was growing on soils, widely contaminated by chlordecone, used in the past as banana plantations. In 2003, French Administration asked for a risk evaluation for the Antilles population. The French Agency for Food Safety, proposed a Provisional Tolerable Daily Intake of 0.0005 mg kg^{-1} b.w. day^{-1}, and an Acute Reference Dose of 0.01 mg kg^{-1} b.w. day^{-1}, based on a toxicological risk assessment. The French National Reference Laboratory for pesticides has carried out two analytical methods, one for food of animal origin and another for food of fruit and vegetable origin. These methods were validated in the reference laboratory and dispatched to 13 laboratories for a proficiency test before the launch of two studies on Martinique and Guadeloupe food. About 900 samples from Martinique were sent to the network of laboratories for analysis of chlordecone. Performance parameters obtained through the proficiency test were briefly reported. The quality control test proposed in this study was discussed to shed light on the true variability achievable by intra-laboratory and inter-laboratory analysis. The limits of conventional quality-control procedures were discussed, and a process was proposed in order to get better confidence in analytical results.

Keywords: Analysis of residues of pesticides; Chlordecone; Foodstuff; Quality control; Interlaboratory analysis

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1. Introduction

Several decades ago, the organochlorine pesticide chlordecone [1,2] was extensively used in the tropics for the control of banana root borer, mainly acting as an effective insecticide against leaf-cutting insects. Chlordecone was used until 1993 in banana plantations of Martinique and Guadeloupe (French Antilles) [3], but it was then prohibited, as its persistence in the environment, sediments, soils, and water, was formally demonstrated [4,5]. The latest surveys conducted in 2001 in French Antilles indeed revealed the presence of chlordecone in soil, rivers, and domestic foodstuffs [6]. Like most pesticide molecules, chlordecone has toxic properties, and exhibits both acute and chronic toxicity [7]. However, if the exposure of the general population through the normal use of chlordecone can be regarded as minimal, the exposure of people living near those plant areas must have been important. Besides, chlordecone bioaccumulates in the food chain and may be found as residues in various foodstuffs. With this background, French Administration decided to carry out a risk assessment to the Antilles population for chlordecone [8]. On the one hand, the French Agency for Food Safety (AFSSA) was asked to provide a Provisional Tolerable Daily Intake (PTDI) and an Acute Reference Dose (ARfD), which were established at $0.0005\,mg\,kg^{-1}\,b.w.\,day^{-1}$, and of $0.01\,mg\,kg^{-1}\,b.w.\,day^{-1}$, respectively [9]. On the other hand, a large study was carried out to determine the exposure of Martinique and Guadeloupe populations to chlordecone in doing the analysis of two series of nearly 1000 food samples each. Sampling included food portions of animal and vegetal origins.

As is widely known, analysis for the detection and quantification of pesticides in complex food matrices, especially the sample preparation and extraction steps, are cumbersome and time-consuming [10,11]. Therefore, the cooperation of a laboratory network is required to perform the analysis of all samples on due time. In this context, the study included, first, the development and formal validation of two analytical methods for chlordecone analysis by the National Reference Laboratory (NRL) for pesticides (our laboratory), followed by the transfer of both methods to a network of 13 analytical laboratories. Before sending samples for chlordecone analysis to the laboratory network, proficiency tests were planned. One of the main concerns for the NRL was to obtain reliable and comparable results from participating laboratories.

The present work describes the development of two analytical methods for chlordecone quantification and their validation by studying their in-house performance. As completion of the analysis of all food samples by the laboratory network was spread over a long period of time, a particular methodology for quality control based on a double analysis of the same samples by participating laboratories and by NRL was implemented as a confirmatory procedure. Moreover, a protocol to correct analytical results taking into account proficiency testing and confirmation data is also described. The different steps of this analytical process are discussed to determine the best way to obtain true values of analytical results produced by a laboratory network in the field of chlordecone analysis in food samples. The present article does not give the chlordecone level in food samples; this is being published as an AFSSA report.

2. Experimental

2.1 *Materials*

A Varian® gas chromatograph (GC) Model 3800 with a 63 Ni electron capture detector (ECD) was used (GC-ECD). The instrument was equipped with a silica capillary column (50 m × 0.32 mm) with a film of 5% phenyl and 95% dimethyl, and 0.25 μm in thickness. The carrier gas was helium delivered at 24 psi. The temperature settings were 50°C for the injection, 300°C for the detector when the column was programmed from 80°C for 2 min to 220°C with a step of 20°C min^{-1}, held at 220°C for 10 min, then to 280°C with a step of 15°C min^{-1}. A Varian® gas chromatograph Model 3800 equipped with a quadrupole (MSMS) 1200 detector was employed. The instrument was equipped with a silica capillary column (30 m × 0.25 mm) with a film of 0.25 μm thickness. The carrier gas was helium delivered at a flow rate of 2 mL min^{-1}. The temperature setting was from 50°C to 250°C with a step of 150°C min^{-1} for the on-column injection. The column was programmed from 80°C held for 1 min, and to 280°C with a step of 20°C min^{-1} and held for 10 min. In these conditions, the chlordecone transitions observed were 272 and 237. The solvents *n*-hexane, dichloromethane, and acetone from Fischer® were pesticide-free. Solutions used were hexane/acetone 85/15 v/v, *n*-hexane/acetone 90/10 v/v, *n*-hexane/diethyl ether 40/60 v/v, and *n*-hexane/diethyl ether 80/20 v/v. Reagents were 0.5 M sodium hydroxide in water from Fischer®; 60% sulfuric acid in water from Merck®. Chlordecone standard (10 mg mL^{-1}) in iso-octane with a purity of 98.5% was purchased from Dr Ehrenstorfer-Schafers®. Solvent evaporation was achieved between 30 and 35°C on a Rotavapor Herdolph® or on a Reacti-Therm Pierce®. Mixing was performed on a Vortex® apparatus. Centrifugation was done on a Hettich® refrigerated system.

2.2 *Method 1: chordecone in food product from animal origin*

A 2 g sample of meat or fish, 4 g of milk or eggs, and 0.5 g of fat were taken. The extraction protocol described previously was carried out in case of an initial extraction step on fat material [12]. The principle is based on 'cold centrifugation extraction', and the extract was mixed with 15 mL of the hexane/acetone 85/15 v/v solvent. The 2 g of meat or fish or 4 g of milk or eggs was blended with 5 mL of hexane/acetone 85/15 v/v and with an internal standard and mixed during 15 s on a Vortex®, then centrifuged for 3 min at 3000 rpm. The organic phase was transferred in a second centrifugation tube, and the solvent extraction step repeated twice with 5 mL of hexane/acetone 85/15 v/v, all solvent extracts being finally combined. A 5 mL sample of the 0.5 M sodium hydroxide solution was added to the extracted solution from fat or from other matrices, mixed slowly for 15 s, and centrifuged 3 min at 3000 rpm. The water phase was removed, and this extraction step was repeated twice with 5 mL of sodium hydroxide solution. The aqueous phases were collected in another tube and washed with 5 mL of hexane. Sulfuric acid solution (5 mL, 60%) was added, and the solution was extracted three times using 5 mL of hexane/acetone 85/15, mixed for 15 s, and centrifuged between each extraction. The final extract was washed with 2 mL of water, and the water phase discarded. The organic phase was evaporated and dissolved in an adequate volume of solvent for gas-chromatographic analysis.

2.3 *Method 2: chlodecone in fruit and vegetable products*

A thoroughly blended sample of 5 g was placed into a 125-mL Erlenmeyer® flask. Acetone (10 mL) was added to cover the sample completely with acetone and with an internal standard then mixed for 2 h. This mixture was filtered on a funnel capped with a piece of wool cotton into a 250-mL separatory funnel. The Erlenmyer® was rinsed twice with 5 mL of acetone transferred on the filter. The sample was pressed on the filter or aspirated to extract acetone completely. The filter was rinsed with 5 mL of acetone, 75 mL of water was added in the separatory funnel, 10 mL of water was saturated with sodium chloride, and then 20 mL of dichloromethane was used for rinsing the Erlenmyer®. The funnel content was mixed for 20 min and stayed until the layers separated, or centrifuged if the separation did not occur. The lower phase was transferred into a 100 mL flask through a funnel containing sodium sulfate. Dichloromethane (20 mL) was poured into the separatory funnel, mixed for 20 min, and transferred into the flask after the layers separated. The funnel was then rinsed with 10 mL of dichloromethane. The dichloromethane was evaporated to 1 mL and transferred in a test tube. The flask was rinsed three times with 1 mL of hexane transferred in the test tube, completely evaporated, and then added to 1 mL of hexane/acetone, 90/10 (v/v). For the clean-up, a 1 g silica cartridge was washed with 5 mL of hexane/diethyl ether 40/60, then 5 mL of hexane/diethyl ether 80/20 (v/v), and finally 5 mL of hexane. The cartridge was not allowed to dry. The extract was transferred on the cartridge and let run by a stopcock at a rate of 1 mL min^{-1} up to the meniscus. After 3 min it was eluted with 5 mL of hexane/diethyl ether 80/20 (v/v). The first 4 mL was discarded, and then the cartridge was eluted with 5 mL of hexane/diethyl ether 40/60 (v/v). The eluate was then evaporated and dissolved in the same solvent as for the standard solution before being injected in the gas chromatograph.

2.4 *Intra-laboratory validation of methods for the determination of chlordecone*

Both methods for the determination of chlordecone were validated according to the standard AFNOR (French Standardization Association) NF V 03-110 [13] and part of the European Decision 2002/657/EN [14]. The limit of detection and limit of quantification for the methods were calculated, and the linearity range determined. The specificity and accuracy were calculated for representative matrices and standard deviation deducted.

2.5 *Food samples*

Two sampling plans were designed by the Department of Risk Assessment of AFSSA, but the present work was done on only one series of 900 food samples, which were collected by the Cellule InterRegionale d'Epidémiologie (CIRE) Antilles-Guyane based in Martinique [15]. The sampling in Martinique included 18 matrices from animal origin and 31 matrices from fruit and vegetable origin, as listed in table 1. Analytical results of the studies are not presented or discussed here, as they are currently published in a separate report by the AFSSA.

Table 1. List of food matrices and number of specimens analysed.

Food samples of animal origin		Food samples of fruit and vegetable origin			
Beef liver	17	Pineapple	4	Turban squash	20
Lamb	8	Eggplant	8	Yam	34
Beef	19	Avocado	20	Lettuce	11
Blood pudding	14	Banana	20	Mango	19
Goat	10	Sugar cane	11	Melon	21
Rabbit	2	Carrot	20	Rutabaga	18
Sheep	10	Cabbage	11	Coconut	17
Mullet	12	Chayote	20	Oignon	20
Eggs	14	Cucumber	34	Water melon	21
Breeding fish	5	Soursop	19	Sweet potato	85
Snapper	10	Zucchini squash	8	Leek	20
Sea bass	12	Dachin	93	Bell pepper	21
Flying fish	8	Meal cassava	23	Ti-nain	24
Tuna	8	Bread fruit	11	Tomato	31
Pork	15	Ginger	8		
Chicken	8				
Sausage	8				

2.6 *Proficiency tests and preparation of test samples*

Proficiency tests for both analytical methods were designed, following the standard NF ISO 13528 [16]. The NRL prepared the samples for the proficiency tests. For method 1, the sample was made from chicken meat as follows: 500 g of mixed chicken meat was spiked at 50 $\mu g\,kg^{-1}$ and split into portions of 2 g. For method 2, 500 g of sweet potatoes was mixed, spiked at 50 $\mu g\,kg^{-1}$, and split into a 5-g portion. Yam and dachin (600 g) were mixed together. A portion of 5 g from the mixture was put into polypropylene bottles and spiked separately at about 50 $\mu g\,kg^{-1}$. For each test sample, 20 individual portions were analysed to test the homogeneity using a *t* test. Robust means were calculated. For this purpose, the standard deviation of the test was chosen as the Horwitz standard deviation for chicken and sweet potato, and as the standard deviation achieved by the LNR in a previous proficiency test for the yam and dachin mixture. The performance of each laboratory was assessed by the *z*-score test. An adjustment step of quality control was activated in case there was any deviation, i.e. if *z*-score was >3. The NRL asked the analytical laboratories to determine the reason for the deviation and advised to help them correct it. If necessary, the NRL can decide to dispatch to participants other batches of unknown samples for analysis (to complete the proficiency study).

2.7 *Quality control*

A particular quality-control procedure was set to test the competency of the analytical laboratories all along the study period. The quality-control plan was composed of about 100 samples representing more than 10% of the total samples to be analysed for the exposure study. Test samples were chosen among samples of the series on pre-determined rules by the reference laboratory. For each laboratory, 50% of the test samples were selected among those being positive (i.e. above the limit of quantification) and those samples with a chlordecone content between the detection limit and

quantification limit. This selection was intended for the diversity of the matrices. The other 50% of quality controls were randomly chosen among samples exhibiting results under the detection limit when using the 'Rand' function of EXCEL®. The list of control samples was set and the samples recalled by the NRL as soon as the analyses were carried out. The NRL analysed the samples using an electron capture detector (ECD) for the screening and tandem mass spectrometry detector (GC/MSMS) for the confirmation. Positive samples up to the limit of quantification (>LOQ) detected by ECD were spiked to check the recovery and analysed by GS/MSMS to confirm the presence of chlordecone. The same analytical sample preparation was used by analytical laboratories and by the NRL. Only the gas-chromatography column and the detector may be different.

3. Results

3.1 *Performance parameters of the methods*

3.1.1 Method 1. The method was validated in the range of 6–80 pg of chlordecone on ECD. The limit of detection was 3 pg, the limit of quantification was 5 pg, and the limits of quantification in samples were $5\,ng\,g^{-1}$ for 2 g of meat or fish sample and $2.5\,ng\,g^{-1}$ for 4 g of milk or egg samples. The specificity was calculated on milk, fish, meat, eggs, and fat by spiking with 25, 50, and $100\,ng\,g^{-1}$ of chlordecone. Student's *t* test did not show any matrix effects. The accuracy was calculated for milk, fish, meat, eggs, and fat. The standard deviation was $7.2\,ng\,g^{-1}$, and the relative standard deviation was 15%.

3.1.2 Method 2. The method was validated in the range of 0–62 pg of chlordecone on ECD. The limit of detection was 3 pg, and the limits of quantification in samples were $2\,ng\,g^{-1}$ for fruits and $5\,ng\,g^{-1}$ for root vegetables. The specificity was calculated on melon, yam, banana, ti-nain, dachin, cucumber, breadfruit, chayote, Caribbean cabbage, and sweet potato, by spiking them with 20, 40, 50, and $60\,ng\,g^{-1}$ of chlordecone. Student's *t* test did not show any matrix effects. The accuracy was calculated on the same sample as for specificity. The standard deviation was $14\,ng\,g^{-1}$, and the relative standard deviation was 8.5%. These results are displayed in table 2.

3.2 *Proficiency testing (PT)*

The PT operates with three different matrices: chicken, sweet potato, and mixed vegetables composed of dachin and yam. The *z*-cores are shown in figure 1 for chicken, figure 2 for sweet potato, and figure 3 for mixed vegetables.

Proficiency test performance parameters were calculated, and the global results are shown in table 3.

Table 2. Performance parameters from the in-house validation for both methods.

Parameters	Method 1	Method 2
Linearity range	6–80 pg	0–62 pg
LOD	3 pg	3 pg
LOQ	5 ng g^{-1} for meat or fish 2.5 ng g^{-1} for eggs or milk	2 ng g^{-1} for fruit 5 ng g^{-1} for root
Specificity and Accuracy in	Level: 25, 50, 100 ng g^{-1} Milk, fish, meat, eggs, fat	Level: 20, 40, 50, 60 ng g^{-1} Melon, yam, banana, ti-nain, dachin, cucumber, breadfruit, chayote, Caribbean cabbage, sweet potato
SD	7.2 ng g^{-1}	14 ng g^{-1}
RSD	15	8.5

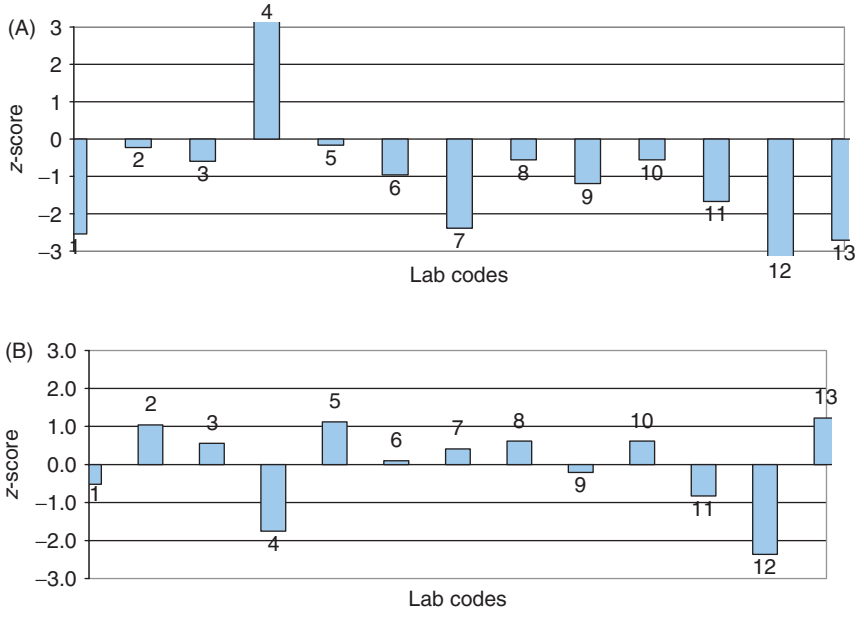

Figure 1. *z*-scores (A) before and (B) after the corrective procedure in a sample of chicken.

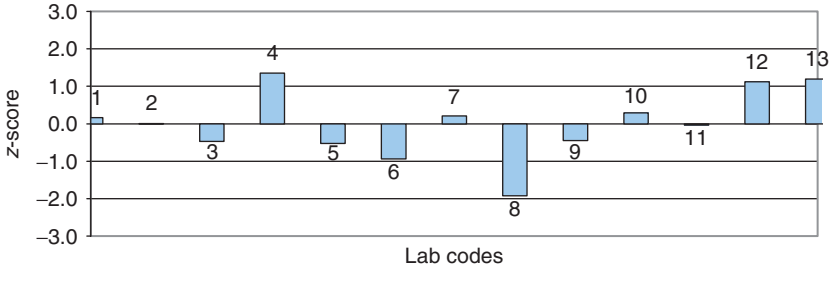

Figure 2. *z*-scores with the sweet potato.

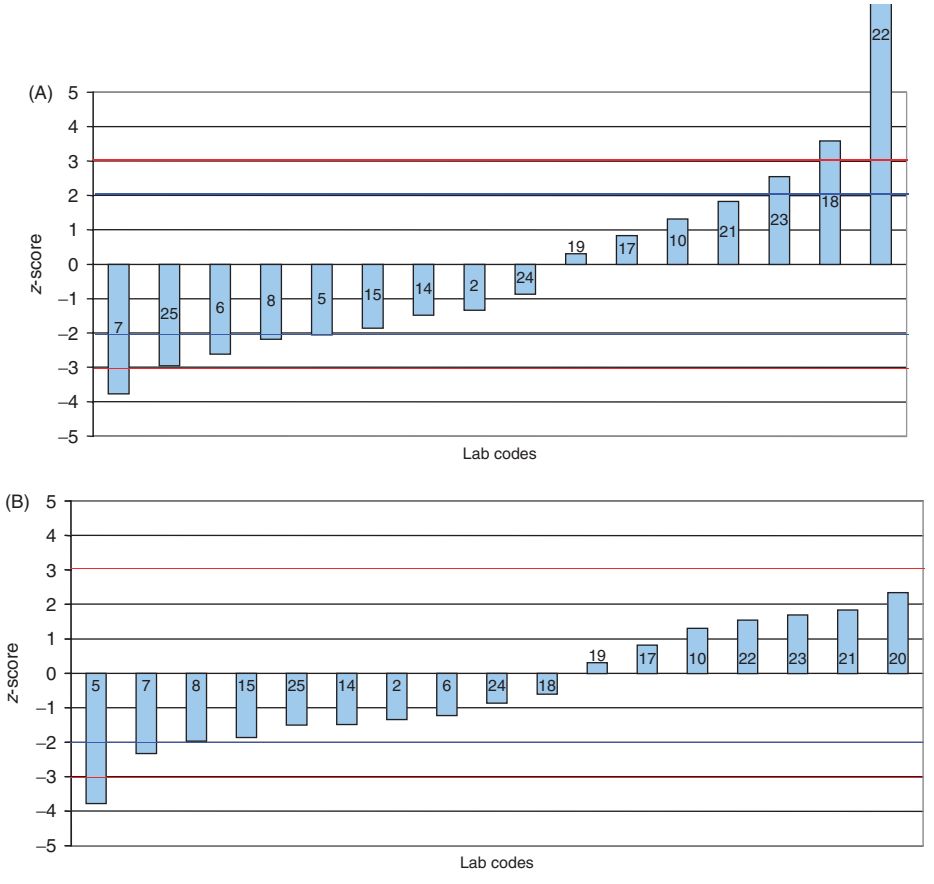

Figure 3. *z*-scores with the mix of yam and dachin (A) before and (B) after the corrective procedure. In (A), the *z*-score was not calculated for laboratory 22 because the variance was too high.

Table 3. Global results of the proficiency testing.

Test	Method	No. of laboratories	Robust mean	SD of the proficiency test	s_r	s_R	RSD
Chicken	1	13	34.5	12.5[a]	–	17	50
Sweet potato	2	13	49	12[a]	–	11	22
Yam + dachin	2	17	26.9	6.5[b]	4.8	18.6	69

[a]Horwitz sd.
[b]Calculated sd.

3.3 *Quality-control results*

The quality-control plan identified 27% false results: 14% false positives and 13% false negatives. The false results details are given in table 4.

The quality-control plan is used to improve the quality of results. After the first conclusion shown in table 5, samples were chosen to test one result or one analytical laboratory. Table 5 gives an example of results achieved after two, three, or four analyses on the same sample by different laboratories.

Table 4. Negative or positive false observed during quality control plan ($\mu g\,kg^{-1}$).

Analytical laboratory	Total no. of samples	No. of control samples	Sample types	Analytical laboratory data	Reference laboratory results[b]	Conclusion
1	46	5	Fish	$5<R<16$	13.8	
			Fish	$5<R<16$	$0.8<R<3.2$	
			Pork	$5<R<16$	$0.8<R<3.2$	LOQ = 16
			Sheep	$5<R<16$	$0.8<R<3.2$	
			Beef	$5<R<16$	$0.8<R<3.2$	
2	81	8	No deviation			
3	70	7	Cucumber	<0.6	5.4	No homogeneity[a]
4	54	5	Dachine	41	<2	No homogeneity[a]
			Ginger	32	<2	
			Sweet potato	7	<3.2	
			Dachine	89	50	
5	92	11	Cucumber	<4	9.6	
6	49	5	Sweet potato	<10	6.9	LOQ = 10
7	48	5	Sweet potato	<10	23.3	LOQ = 10
8	114	5	Sweet potato	<3	76.9	No homogeneity[a]
			Cucumber	<3	5	
9	50	0	No confirmation			
10	61	6	No deviation			
11	74	8	Dachine	88	<3.2	
			Rutabaga	12.3	<3.2	
			Dachine	<3	35.7	
			Sweet potato	110	<3.2	
12	68	12	No deviation			
13	78	5	Water melon	2.5	<0.8	No homogeneity[a]
			Yam	<0.8	$0.8<R<3.2$	
			Tomato	<2	<0.8	
			Carrot	<0.8	$0.8<R<3.2$	

[a]The first analysis and the confirmation analysis were identified as not applied on the same sample.
[b]R was the result.

For six samples, it was not possible to explain the observed deviation despite additional analysis by participants or by mass spectrometry by the reference laboratory (table 6). Discrepancies among data will be discussed below.

4. Discussion

4.1 *Method 1*

This method was developed according to Blanke *et al.* [17]. The skill of the method was to chemically transform chlordecone in a water-soluble compound so that the fatty material could be easily separated from the water phase to obtain a very clean extract. The acidic step was also very powerful in destroying lipids and a many other undesirable compounds. This method was relevant for all fatty matrices from animal origin listed in table 1 and was validated for meat, milk, fat, fish and egg. It was used also for fruits and vegetables loaded in fat, like avocados and coconuts for which the method was working successfully. The performance parameters in the method were in the range of the usual performance for pesticides [18].

Table 5. Example of discrepancies among analytical data from analytical and reference laboratories.[a]

Sample	Analytical laboratory result	Reference laboratory result	1st conclusion	1st control result	2nd control result	2nd conclusion
Cucumber 1	3	<3.2	OK	<3	<2	OK
Cucumber 2	6	5	OK	25	11	OK but no homogeneity
Dachin 1	20	17	OK	45	54	OK but no homogeneity
Dachin 2	<3	36	False −ve			MSMS
Dachin 3	89	50	OK			
Rutabaga	4	9	OK	4	7	OK
Cabbage	<1	<3.2	OK	<3	<2	Trace OK
Ginger	32	<2	False +ve	<3		Trace, MSMS
Sweet potato	12	<3.2	False +ve			<3.2, MSMS
Fish	<16	<3.2	LQ = 16			<3.2, MSMS

[a]LQ: limit of quantification. MSMS: tandem mass spectrometry. 1st and 2nd control results performed by two analytical laboratories.

Table 6. Samples where no explanation was found for discrepancies among analytical results.

Sample type	Ref. no.	First analysis[a] ($\mu g\,kg^{-1}$)	Confirmation[b] ($\mu g\,kg^{-1}$)
Dachin	3	88	nd[c]
Sweet potato	5	110	nd
Dachin	112	41	nd
Dachin	363	nd	36
Sweet potato	441	nd	77
Sweet potato	621	76	541

[a]Done by participants.
[b]Done by the reference laboratory.
[c]nd: not detected, or below the limit of detection.

4.2 *Method 2*

The analytical method developed for food of fruit and vegetable origin was a very common protocol for pesticides. It proceeds with acetone extraction, liquid–liquid separation, and SPE clean-up. This method was relevant for all fruits and vegetables matrices listed in table 1. Nevertheless, the method did not work well for ginger and for some other matrices like avocado and coconut for which method 1 was indicated. For ginger, the additional acidification did not clean up enough of the extract for an ECD analysis. The MS or MSMS analysis should then be used.

4.2.1 Proficiency test (PT). The aim of these PTs was to qualify a network of laboratories able to analyse the samples for chlordecone and to gain some confidence in the results so that they could be valuable for risk-assessment studies. The NRL wrote a quality manual and a quality procedure to implement a formal PT scheme based on the ISO 17025 standard. The choice of statistical PT tests was previously discussed by a working group led by the NRL for pesticides. As method 1 requires a large amount of handling, it was perceived by participants as difficult to apply. It was stressed that analysts should be well trained to handle it. Five laboratories exceeded the z-score of 2, and one of them could not be lowered even after the corrective test (figure 1).

The proficiency test demonstrated that laboratory no. 12 did not succeed in working with the method 1. This laboratory was finally not selected to run the analysis of food of animal origin by this method in the study. As shown in table 3, a relative standard deviation (RSD_R) of 22% was obtained on the first run with sweet potatoes with method 2. This satisfactory RSD_R demonstrated that method 2 was easy to achieve by participants even if they were not specially trained for it (figure 2). However, this result did not reflect the true performance of the method when used in a routine analysis. In fact, some problems may come into sight when a large number of different and unknown matrices were analysed. As indicated previously, method 2 was not suitable for the fatty matrices. The PT for mix of yam and dachin gave some outliers (figure 3). Three laboratories were outliers with a *z*-score above 3. The second run after the corrective test improved the performances, but one laboratory remains with a *z*-score above 3 and two other laboratories above 2. These last laboratories were not kept for the analysis of food samples because the level of confidence needed for risk-assessment studies could not be reached. It is also interesting to compare performance criteria obtained through the in-house validation by the NRL (table 2) to the global performances of the network through the proficiency testing (table 3). The RSD obtained through the in-house validation was 15 for method 1 and 8.5 for method 2. The RSD resulting from the proficiency test was 50 for method 1 and 22/69 for method 2. Even though the RSD obtained from the performance among the laboratories is worse than that obtained from the single laboratory (NRL) that set up the method, it is noteworthy that planning Proficiency Tests will help laboratories to check their performances which are expected to improve with growing experience with the method adopted.

4.2.2 Quality control. According to our quality-control programme, the plan was to test 10% of the samples to confirm the results of the analytical laboratories. The NRL anticipated that the PT was not sufficient to gain confidence in analytical laboratory results. The first reason was that participants often considered PT as a very specific exercise, and samples were then often not analysed as simple routine samples; considerable skill is involved in analysing the samples. The second reason was that the validation of the method and PT could not be applied on each matrices of the sampling plan. When the validation of the methods and the proficiency tests are running, the NRL may not exactly know the composition of the sampling plan. For instance, in our case, the Risk Assessment Direction of AFSSA generated the sampling plan after using the results from the consumer survey. The choice of matrices for validation and PT was pointed on the notorious contaminated samples and consumed food. The number of consumed foods was very high, and the NRL was not practically able to process each of the 50 matrices in the validation procedure and in the PT. The selection of matrices was stepped up on animal matrices, as chlordecone is a lipophilic compound and on root vegetable, as chlordecone was found at high levels in these matrices. The expectation was to be able to extrapolate validation and PT results with the quality control, mainly for the methods to be able to analyse the matrices. It was demonstrated that method 2 was unable to analyse fatty vegetables matrices. Hopefully, method 1 could work for this kind of matrix. Some analytical deviations coming from the practice of the methods by laboratories and coming from the variability among the fifty kinds of matrices of the sampling plan (table 1) could unfortunately be expected.

However, as laboratories 2 and 12 (table 4) found no deviation and obtained good results after PTs, the 20 samples analysed by these laboratories and confirmed by the NRL were considered a reference to test other laboratories. Both laboratories were considered as confident laboratories.

As mentioned before, the results showed some false negatives and positives (table 4). Different courses of action were taken to gain a better understanding of the results and to determine how to correct deviations. First of all, a excessively high quantification limit may explain the observed deviation for three laboratories (nos 1, 6, and 7). One of them was able to improve its quantification limit, but the two others did not. The results of those latter laboratories were finally not accepted, and the NRL should analyse the 86 samples for these two laboratories. Second, analytical laboratories were again asked to correct their false results. For this purpose, each laboratory with displayed false positive or negative results received a new specific lot of reference samples to analyse. This lot of reference samples was confirmed by at least two laboratories having displayed no false results. The new set of analytical results obtained after this test was good enough to qualify the analytical quality of the laboratories.

The correspondence with the reference results showed evidence of the ability of the analytical laboratories to yield good results if they are alerted (table 5), i.e. in a range of control and not of routine analysis. In such a case, the agreement among different analytical results in table 5 for cucumber 1, rutabaga, and cabbage was perfect. The uncertainty in the chlordecone analysis was about 30% [19]. Within that limit, the results for rutabaga of 4, 9, 4, and 7 $\mu g\,kg^{-1}$ may be considered reasonably equivalent, considering that there is always an unknown uncertainty upon the homogeneity of the sample. For cucumber 2 and dachin 1, the four results are in qualitative agreement, but the results greatly exceeded the target value with the uncertainty limit of 30%. In that case, it was estimated that the sample was not homogenous as a survey confirmed, and later on a survey proved this case. For ginger and sweet potato, positive results were found by the analytical laboratory but not by the NRL: In the case of ginger, the control was achieved by the NRL by using the ECD detector and MSMS detector and also by another analytical laboratory which used an MS detector. Finally, the result was below the limit of quantification with the presence of chlordecone above the limit of detection. The first result for fish (<16) was not considered correct because the limit of quantification was too high ($LQ = 16$). The laboratory which produced this result did not agree to carry on the analysis of food samples because it was not able to improve its limit of quantification. The result of dachin 2 is a false negative because the mass spectrometry detector detected the transitions 272 and 237 of chlordecone. Both results of dachin 3 were in agreement inside the interval of the uncertainty limit of 30%. Finally, the results were in agreement, as they were qualitatively and quantitatively confirmed. In case of qualitative difference, i.e. a discrepancy among two laboratories, the analytical data from the NRL or from a confident laboratory were only taken into account.

Therefore, several results were quite different between laboratories, as can be seen in table 6. For six samples, it was not possible to endorse only one set of data. These six samples were in fact obtained on sweet potatoes and dachin only. These vegetables were roots in contact with the ground, known as often contaminated, and not easy to blend without a suitable material, producing a non-homogeneous mixture. In this case,

it could be stated that results produced by different laboratories are weakly comparable. More likely, it may be possible that sweet potatoes and dachin accumulate chlodecone in a specific zone. The recommendation was to grind the whole sample for the preparation of the samples for such matrices. A survey revealed that several laboratories did not do this, and consequently it would be assumed that the samples analysed were not the same for the various laboratories. This was due to the absence of a sample preparation protocol in the method provided, in spite of a formal recommendation.

5. Conclusion

This article describes two methods for the analysis of chlordecone respectively applicable to food of animal origin and food of fruit and vegetable origin. Both methods were validated, and the proficiency testing was settled to recruit a network of analytical laboratories. Analytical results were checked by a confirmation plan. The results were checked and/or accepted or not with specific procedures. This article has discussed how to improve analytical results through a network of laboratories. The results indicate that the accreditation of laboratories under the standard ISO 17025, and the validation of methods as well as the proficiency testing, were not enough to obtain absolute confidence in the results. The level of the requirement was very high for the analytical laboratories because one of the objectives of this study was to determine the impact on the public health in French Antilles. We tried to improve this quality procedure. It was obvious that PT showed the best result of a laboratory, but unfortunately, this is not always the same during routine analysis. It is sometimes observed that laboratories have a propensity to exercise the greatest care when analysing PT materials than with routine samples. This work aims to point out the aspects that lead to the greatest confidence in analytical results. This requirement is an absolute need for being able to transfer the analytical results for risk assessment.

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