CIPAC 4828/R

MT 19X WASH RESISTANCE INDEX OF LN

Small Scale Collaborative Trial

REPORT TO CIPAC

Olivier Pigeon, Walloon Agricultural Research Centre (CRA-W), Belgium
Markus Müller, Agroscope Changins-Wädenswil Research Station ACW, Switzerland
Yumiko Kozuki, Sumitomo Chemical Co., Ltd, Japan
Martin Rodler, Syngenta, Switzerland

June 2012

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1 Participants

Five participants from five countries took part at the small scale collaborative trial. Participating laboratories are listed in alphabetical order whereas lab numbers in the result tables and graphs were assigned, chronologically, based upon receipt of results.

Laboratory	Name
Agroscope Changins-Wädenswil Research Station ACW Federal Department of Economic Affairs DEA Research Department Food Quality and Safety, Nutrition and Health Research Group Plant Protection Chemistry Schloss 1, Postfach 8820 Wädenswil SWITZERLAND	Markus D. Müller Bruno Patrian
Bayer Environmental Science Agricultural Center Monheim Alfred Nobel Str. 50 Building 6820 40789 Monheim GERMANY	Ronald Stoecker
Sumitomo Chemical Co., Ltd Health & Crop Sciences Research Laboratory Formulation Development Group 4-2-1, Takatsukasa, Takarazuka, Hyogo, 665-8555 JAPAN	Yumiko Kozuki
Vestergaard Frandsen SA Vestergaard Frandsen Vector Control Laboratories 253/9 MINHKHAI Hai Ba Trung Hanoi VIETNAM	Chi Phan
Walloon Agricultural Research Centre (CRA-W) Agriculture and Natural Environment Department Plant Protection Products and Biocides Physico-chemistry and Residues Unit Rue du Bordia, 11 B-5030 Gembloux BELGIUM	Olivier Pigeon

The Walloon Agricultural Research Centre (CRA-W) was the coordinator of the Small Scale Collaborative Trial : preparation and sending of the LN samples, the analytical standards and the necessary documentation to the participating laboratories, compilation of results, interpretation of results and preparation of the draft report.

2 LN samples

- Interceptor®: alpha-cypermethrin 6.7 g/kg long-lasting (coated onto polyester) insecticidal mosquito net (LN), multifilament, 75 denier.
  [WHO interim specification 454/LN/1 (October 2009)].
  Supplier : BASF.
- LifeNet® : deltamethrin 8.5 g/kg long-lasting (incorporated into polypropylene) insecticidal mosquito net (LN), multifilament, 100 denier.
  [WHO interim specification 333/LN/4 (September 2011)].
  Supplier : Bayer Environmental Science.
- Olyset® : permethrin 20 g/kg long-lasting (incorporated into polyethylene) insecticidal mosquito net (LN), monofilament, 150 denier.
  [Amended WHO interim specification 331/LN (July 2006)].
  Supplier : Sumitomo Chemical Co., Ltd.
- PermaNet® 2.0 : deltamethrin 1.4 g/kg long-lasting (coated onto polyester) insecticidal mosquito net (LN), multifilament, 100 denier.
  [WHO interim specification 333/LN/1 (NETTING) (September 2010)].
  Supplier : Vestergaard Frandsen.

3 Analytical standards

- Alpha-cypermethrin, certified analytical standard of known purity (99.4%),
  Supplier : BASF.
- Deltamethrin, certified analytical standard of known purity (99.6%),
  Supplier : Bayer CropScience.
- Permethrin 40:60 *cis:trans,* certified analytical standard of known purity (97.4%),
  Supplier : Sumitomo Chemical Co., Ltd..

4 Study protocol

4.1 Preparation of LN samples

Performed by the Walloon Agricultural Research Centre (CRA-W).

From each LN sample (4), 6 times 3 pieces of 25 cm x 25 cm (1 piece from each large side and 1 piece from the roof) were cut with scissors from the entire net for determination of wash resistance index after 0, 1, 2, 3, 4 and 5 washes (3 pieces for each wash cycle). The 3 pieces of each wash cycle were put into an aluminium foil and identified with the LN name and the wash cycle.

LN	Wash cycle	Net pieces
Interceptor® (Mo 120)	0 W	3 individual pieces
	1 W	3 individual pieces
	2 W	3 individual pieces
	3 W	3 individual pieces
	4 W	3 individual pieces
	5 W	3 individual pieces
LifeNet® (Mo 118)	0 W	3 individual pieces
	1 W	3 individual pieces
	2 W	3 individual pieces
	3 W	3 individual pieces
	4 W	3 individual pieces
	5 W	3 individual pieces
Olyset® (Mo 111)	0 W	3 individual pieces
	1 W	3 individual pieces
	2 W	3 individual pieces
	3 W	3 individual pieces
	4 W	3 individual pieces
	5 W	3 individual pieces
PermaNet® 2.0 (Mo 112)	0 W	3 individual pieces
	1 W	3 individual pieces
	2 W	3 individual pieces
	3 W	3 individual pieces
	4 W	3 individual pieces
	5 W	3 individual pieces

This procedure was performed for each laboratory (5). A total of 18 net pieces of 25 cm x 25 cm from each LN were then cut and sent by express courier to each laboratory together with 500 / 2000 mg of analytical standard.

The total number of pieces of 25 cm x 25 cm which were prepared were:
- 5 laboratories
- 4 LNs
- 6 wash cycles
- 3 pieces per wash cycle

TOTAL = 360 pieces of 25 cm x 25 cm

4.2 Sending of samples to participants

Performed by the Walloon Agricultural Research Centre (CRA-W).

Each participating laboratory received:

- The net pieces of Interceptor®, LifeNet®, Olyset® and PermaNet® 2.0 (18 net pieces per LN) to be used for the determination of the wash resistance index according to the CIPAC washing method MT 19X.
- An additional piece of each LN (corresponding to ¼ of entire net) for any preliminary trial on washing method or active ingredient content method, if desired.
- Certified analytical standards of alpha-cypermethrin (500 mg), deltamethrin (500 mg) and permethrin 40:60 *cis: trans* (2000 mg).
- All the documentation needed for the Collaborative Trial:
  - The protocol of the CIPAC Small Scale Collaborative Trial.
  - The CIPAC method MT 19X (wash resistance index of LN).
  - A copy of the analytical methods to be used for deltamethrin content in LifeNet® and PermaNet® 2.0. The methods for alpha-cypermethrin content in Interceptor® and permethrin content in Olyset® are published in the CIPAC Handbook M.
  - The Certificate of Analysis (CofA) and Material Safety Data Sheet (MSDS) of the analytical standards.

The excel file to be filled for the reporting of results was also sent by E-mail.

4.3 Method for determination of wash resistance index

CIPAC method MT 19X : WASH RESISTANCE INDEX OF LN

[3 determinations per wash cycle = 3 pieces per wash cycle]

See CIPAC method MT19X (CIPAC 4827/m)

- Washing of 1 individual piece of 25 cm x 25 cm with 500 mL of a 5 g/L CIPAC washing agent solution at 30°C ± 2°C by inverting the 1 L bottle 10 times and then by standing for 10 minutes at 30°C ± 2°C.
- Rinsing 2 times with 500 mL deionized water at 30°C ± 2°C by inverting the 1 L bottle 10 times and then by standing for 10 minutes at 30°C ± 2°C (After rinsing, remove the net sample, replace the deionized water, insert the sample into the 1 L bottle and repeat this rinsing step).
- Drying on a line at ambient temperature protected from direct sunlight for 30 minutes and then at 40°C ± 2°C in a closed glass bottle for a time period of 22 ± 2 hours before the next washing.
- After the wash cycles, storage of the piece into a glass bottle or in an aluminium foil in a refrigerator at 4°C (± 3°C).

Determination of active ingredient content

After the washing procedure, determination of active ingredient content in each individual piece [1 determination for each individual piece and duplicate chromatographic injections]. If possible, analyse all samples at the same time. Results were expressed as g active ingredient per kg net material (to the nearest 0.01 g/kg). The mean and RSD of the 3 pieces for each wash cycle were calculated and the wash resistance index (expressed as percentage to the nearest 1%) was calculated for each wash cycle using the equation for a free migration stage behaviour

Methods for active ingredient content

- Interceptor®: CIPAC method 454/LN/M/3.1 (alpha-cypermethrin in coated nets), CIPAC Handbook M, page 40.
- LifeNet®: provisional CIPAC method for the determination of the total content of deltamethrin in incorporated into polypropylene LN (CIPAC 4797/m).
- Olyset® : CIPAC method 331/LN/M/3 (permethrin in incorporated nets), CIPAC Handbook M, page 159.
- PermaNet® 2.0 : CIPAC method for the determination of deltamethrin in coated nets (CIPAC 4497/m).

5 Comments from the participants

Participants reported deviations from the CIPAC washing method or from the methods used to determine the active ingredient content and provided comments and suggestions on the CIPAC washing method.

5.1 Laboratory 1

Deviations from CIPAC analytical methods for active ingredient content

Interceptor®

The internal standard was added before to reflux at 90°C for 5 minutes instead of after.
The spit ratio of the injection system is 10:1 instead of 75-100:1.
The method was successfully validated for specificity, linearity of detector response, accuracy and repeatability and is ISO 17025 accredited.

LifeNet®

No change.
The method was successfully validated for specificity, linearity of detector response, accuracy and repeatability.

Olyset®

No change.
The method was successfully validated for specificity, accuracy and repeatability and is ISO 17025 accredited.

PermaNet® 2.0

The samples were extracted with 24 mL of isooctane / dioxane (80/20, v/v) instead of 14 mL and the calibration curve was slightly adapted to bracket the concentration in the sample solutions.
HPLC column: Phenomenex Luna CN, 5 µm, 250 x 4.6 mm (instead of Lichrosorb Si60, 5 µm, 150 x 4.6 mm).
Detection at 230 nm instead of 236 nm

The method was successfully validated for specificity, linearity of detector response, accuracy and repeatability and is ISO 17025 accredited.

Deviations from the CIPAC washing method

The CIPAC washing method was applied exactly as described.

Comments on the CIPAC washing method

The wash method is straightforward and easy to carry out. If the washing and rinsing operations are well planned and organized, the method permit to perform the washing and rinsing of 36 net pieces (12 samples in triplicate) in a half day with 2 technicians.

5.2 Laboratory 2

1. There are change in analytical method used for analysing Interceptor and Olyset samples. The change was communicated with Dr Oliver Pigeon and got approval to continue. Outline of methods is given below.

   Interceptor: alpha-cypermethrin 6.7 g/kg long-lasting (coated onto polyester)
   Outline of method: Alpha-cypermethrin is extracted from the net by mixture of solvent of n-hexane and 1,4-dioxane (95: 5, v:v) with internal standard of dibutylphthalate added. A mixture is then shaked and sonicated to extract alpha-cypermethrin. Extracted solution is filtered through 0.45 μm Teflon© membrane and then analyzed by a normal phase high performance liquid chromatography at 236 nm for Alpha-cypermethrin concentration. The precision as RSDr and the recovery of the method are 0.79% and 101%, respectively.

   Olyset: permethrin 20 g/kg long-lasting (incorporated into polyethylene)
   Outline of method: Permethrin contained PE and PP sample is added xylene as an extraction solvent and spiked with deltamethrin as an internal standard. Permethrin is extracted in boiling xylene. The extraction solution is dried, reconstituted in mixture of n-hexane and 1,4-dioxane (95+5, v/v) as mobile phase and filtered by 0.45 μm Teflon© membrane. Permethrin in the filtered solution is determined by a normal phase high performance liquid chromatography at 236 nm. The precision as RSDr and the recovery of the method are 1.75% and 102%, respectively.

2. We followed exactly the methods given for Lifenet and PermaNet.
3. Observations:
   a. To run the test for 4 bednet samples received, it takes full 15 man day only for washing.
   b. The washing cycle is likely prone to human error. Monotonous actions may impact negatively the technician’s working discipline.

5.3 Laboratory 3

Comments Interceptor

Deviations of active ingredient content method:
We filtered the extracts through a 0.45 µm Teflon filter.
We dissolved a 10 % solution of citric acid in THF (instead of 5 %).
Our autosampler tray cannot be cooled, so we used room temp instead of 15°C. The entire sample sequence took 30 hrs, which corresponds to the max time of the last sample before injection.

Deviations of washing method:
We used a thermostatted oven instead of a vibration-free water bath. The bottles were thermostatted overnight, and just removed from the oven for the inversions.

Suggestions:
The wash method is straightforward and easy to carry out. The use of a thermostatted oven at 30 ± 2 °C should be included as an option for laboratories not having access to a vibration free water bath.

Comments LifeNet

Deviations of active ingredient content method:
Column used: Nucleosil 100-5-CN, 250 x 4mm, 5µm (instead of Phenomenex Luna CN 100 A, 250 x 4.6mm, 5µm).
Retention times: 3.3 min for dibutyl phthalate and 5.1 min for deltamethrin (instead of 4-5 min und 8-9 min, respectively).
Filters: 0.45 µm PTFE Filter instead of 0.2 µm nylon.
Regarding evaporation of sample extracts, we used the alternative described as": *Another alternative is to introduce 0.5 ml of the filtered extract into a 1.5 ml chromatographic vial and to evaporate the solvent at ambient temperature to nearly dryness under a stream of dry nitrogen (about 1ml/min)"* but used 30 °C.

Deviations of washing method:
We used a thermostatted oven instead of a vibration-free water bath. The bottles were thermostatted overnight, and just removed from the oven for the inversions.

Suggestions:
The wash method is straightforward and easy to carry out. The use of a thermostatted oven at 30 ± 2 °C should be included as an option for laboratories not having access to a vibration free water bath.

Comments Olyset

Deviations of active ingredient content method:
The pretests revealed that the pieces were not entirely covered by the extraction solvent. Therefore, the sample size was reduced to 1 g instead of 2 g.
Standard solutions were prepared with half concentrations to compensate for smaller sample size.
Furthermore, 50 instead of 100 ml of standard was prepared, having the advantage that same volume of internal standard could be added (minimizing errors generated by changing pipettors and tips). Standard solutions were prepared in 50 ml volumetric flasks instead of Duran bottles. And obviously, the factor of 2 was omitted in the calculus.

Deviations of washing method:
We used a thermostatted oven instead of a vibration-free water bath. The bottles were thermostatted overnight, and just removed from the oven for the inversions.

Suggestions:
The wash method is straightforward and easy to carry out. The use of a thermostatted oven at 30 ± 2 °C should be included as an option for laboratories not having access to a vibration free water bath.

Comments PermaNet 2.0

Deviations of active ingredient content method:
HPLC column: Nucleosil 100-5-CN, 250 x 4mm, 5µm (instead of Lichrosorb Si60, 5µm, 150x4.6).
Retention times 3.5 min for dipropyl phthalate and 5.1 min for deltamethrin.
We do not have a thermostatted ultrasonic bath, so we used 85°C water in our bath. Exchange of water repeated every 5 min (3 x 5 min equals 15 min immersed in ultrasonic bath).
Shaking frequency 200 beats/min (range in method 150-200 beats).

Deviations of washing method:
We used a thermostatted oven instead of a vibration-free water bath. The bottles were thermostatted overnight, and just removed from the oven for the inversions.

Suggestions:
The wash method is straightforward and easy to carry out. The use of a thermostatted oven at 30 ± 2 °C should be included as an option for laboratories not having access to a vibration free water bath.

5.4 Laboratory 4

Analytical method and conditions

Interceptor®

GC Equipment

Type of Gas Chromatograph: Shimadzu GC-2010
Type of Integrator: Shimadzu flame monitor FLM-2

GC Conditions

Column: fused silica, 0.32 mm (i.d.) × 30 m, film thickness: 0.25 µm, coated with crosslinked dimethyl polysiloxane, Agilent Technologies J&W DB-1
Column Temperature: 225°C
Injection Temperature: 260°C
Detector Temperature: 300°C
Carrier Gas: helium
Flow Rate: 33.5 cm/s
Detection: flame ionization
Injection System: split injection
Split Ratio: 1/75
Injection Volume: 2 µl
Mode: automatic
Peak Measurement: area

LifeNet®

HPLC Equipment

Type of Liquid Chromatograph: Shimadzu LC-6A
Type of Integrator: Shimadzu C-R3A

HPLC Conditions

Column: stainless steel, 250 x 4.6 (i.d.) mm, packed with Zorbax CN (5 mm)
Column Temperature: 35°C
Mobile Phase: *iso*-octane/tetrahydrofuran = 93/7 (v/v)
Flow Rate: 1.0 ml/min
Detector Wavelength: 230 nm
Injection Volume: 20 µl
Mode: automatic
Peak Measurement: area

Olyset® Net

GC Equipment

Type of Gas Chromatograph: Shimadzu GC-2010
Type of Integrator: Shimadzu flame monitor FLM-2

GC Conditions

Column: fused silica, 0.25 mm (i.d.) × 30 m, film thickness: 0.25 µm, coated with crosslinked dimethyl polysiloxane, Agilent technologies DB-1
Column Temperature: 240°C
Injection Temperature: 265°C
Detector Temperature: 265°C
Carrier Gas: helium
Flow Rate: 30 cm/s
Detection: flame ionization
Injection System: split injection
Split Ratio: 1/100
Injection Volume: 1 µl
Mode: automatic
Peak Measurement: area

PermaNet® 2.0

HPLC Equipment

Type of Liquid Chromatograph: Shimadzu LC-6A
Type of Integrator: Shimadzu C-R3A

HPLC Conditions

Column: stainless steel, 250 x 4.6 (i.d.) mm, packed with Zorbax CN (5 mm)
Column Temperature: 35 °C
Mobile Phase: *iso*-octane/tetrahydrofuran = 93/7 (v/v)
Flow Rate: 1.0 ml/min
Detector Wavelength: 230 nm
Injection Volume: 20 µl
Mode: automatic
Peak Measurement: area

Deviations from CIPAC LN analytical method

Interceptor®

Though it was written that the sample solutions should be prepared in triplicate for each sample, two sample solutions were prepared because of shortage of the sample. Two values of extremely high or low active ingredient content measured were excluded (the Smirnov-Grubbs outlier test ($P < 0.01$)).

LifeNet®

The mobile phase and the extraction solvent were substituted from 1,4-dioxane to tetrahydrofuran because 1,4-dioxane is classified as a designated chemical substance in our country. Sample preparation: Samples were heated up in a hot water bath instead of under reflux. (The volumetric flask was heated up at approximately 80 °C for 30 minutes until propylene nets are completely dissolved.)

Olyset® Net

Though it was written that the sample solutions should be prepared in duplicate for each sample, one sample solution was prepared because of shortage of the sample. (25 × 25 net sample = about 2 g [About 40 mg of permethrin is contained in it.])

PermaNet® 2.0

The mobile phase and the extraction solvent were substituted from 1,4-dioxane to tetrahydrofuran because 1,4-dioxane is classified as a designated chemical substance in our country.

Deviations from the CIPAC washing method

The way of fold was changed during heating process as follows: “Fold the sample carefully twice in each direction, place it loosely rolled in a glass bottle.”

Comments and suggestions on the CIPAC washing method

How to fold samples

I suggest to change the draft sentence from “once” to “once or twice” because it is more feasible. (page 2, paragraph (d) Heating)
From; Fold the sample carefully once in each direction…
To; Fold the sample carefully once or twice in each direction…
(The proper number of times depends on the glass bottle size. 100 ml glass bottle -> twice, 125 ml glass bottle -> once)

CIPAC washing agent

It was applicable for permethrin, deltamethrin and alpha-cypermethrin.

Comprehensive evaluation

It seemed that this method was applicable for coated as well as incorporated LNs with different active ingredients and polymers. However, it premises on the good and homogenized LNs. The active ingredient content range measured on coated LN tends to more distributed. The accurate AI analytical method is absolutely essential.

5.5 Laboratory 5

LifeNet/Deltamethrin

No changes.

Permanet/Deltamethrin

The column size used was **250** x 4,6mm (method “Method deltamethrin in PermaNet 2.0” describes column LiChrosorb SI60 5µm **150** x 4,6mm).

Olyset/Permethrin

The column size used was 30m x **0,32 mm** with 0,25µm film thickness (method CIPAC/4503/m describes column DB-1, 30m x **0.25 mm** with 0,25µm film thickness).

Interceptor/Cypermethrin

Volume of citric acid was defined with **30 µL** via pipette to obtain a defined and reproducible volume for all samples measured by GC (CIPAC Handbook M, page 40 describes „**one drop** of citric acid“).

To reduce the gas volume within the vials, the sample volume of 200 µL was filled in a vial with insert.

6 Results from participants

6.1 Laboratory 1

LN	Wash	Active ingredient content (g/kg)					a.i. wash resistance	Average a.i. wash resistance
		A (*)	B (*)	C (*)	Mean	RSD (%)	(% of wash 0)	(% at each wash) (**)
Interceptor®	0	5.92	5.78	6.33	**6.01**	4.8	**-**	**-**
	1	5.59	~~8.45~~	6.04	**5.82**	5.5	**96.8**	**96.8**
	2	5.46	5.94	5.22	**5.54**	6.6	**92.2**	**96.0**
	3	5.63	~~8.14~~	5.29	**5.46**	4.4	**90.8**	**96.9**
	4	5.45	6.83	5.16	**5.81**	15.3	**96.7**	**99.2**
	5	5.32	6.22	4.76	**5.43**	13.6	**90.4**	**98.0**
LifeNet®	0	13.12	11.93	12.31	**12.45**	4.9	**-**	**-**
	1	10.08	9.81	10.51	**10.13**	3.5	**81.4**	**81.4**
	2	9.06	9.25	8.78	**9.03**	2.6	**72.5**	**85.2**
	3	8.70	9.14	8.68	**8.84**	2.9	**71.0**	**89.2**
	4	7.62	7.85	7.91	**7.79**	2.0	**62.6**	**88.9**
	5	7.06	7.09	6.54	**6.90**	4.5	**55.4**	**88.9**
Olyset®	0	19.96	20.24	19.79	**20.00**	1.1	**-**	**-**
	1	19.61	19.79	19.97	**19.79**	0.9	**99.0**	**99.0**
	2	19.53	20.03	20.08	**19.88**	1.5	**99.4**	**99.7**
	3	19.63	19.53	19.75	**19.64**	0.6	**98.2**	**99.4**
	4	19.51	19.53	19.69	**19.58**	0.5	**97.9**	**99.5**
	5	19.89	19.47	19.20	**19.52**	1.8	**97.6**	**99.5**
PermaNet® 2.0	0	1.40	1.19	1.27	**1.29**	8.2	**-**	**-**
	1	1.07	1.21	1.15	**1.14**	6.1	**88.9**	**88.9**
	2	1.32	1.05	1.11	**1.16**	12.2	**90.2**	**95.0**
	3	1.14	1.09	1.10	**1.11**	2.4	**86.3**	**95.2**
	4	1.03	1.03	~~1.86~~	**1.03**	0.0	**80.1**	**94.6**
	5	1.04	0.99	~~1.81~~	**1.02**	3.5	**78.9**	**95.4**

(*) Each result is the mean of 2 chromatographic injections (duplicate injections).

(**) $w = 100 \times \sqrt[n]{(t_n/t_0)}$

where:

t_n = total active ingredient content (in g/kg) after n washing cycles. This value is calculated by averaging the total active ingredient content of the 3 pieces.

t_0 = total active ingredient content (in g/kg) before washing (no washing). This value is calculated by averaging the total active ingredient content of the 3 pieces.

w = wash resistance index, expressed as percentage.

6.2 Laboratory 2

LN	Wash	Active ingredient content (g/kg)					a.i. wash resistance	Average a.i. wash resistance
		A (*)	B (*)	C (*)	Mean	RSD (%)	(% of wash 0)	(% at each wash) (**)
Interceptor®	0	6.57	6.08	7.56	**6.74**	11.19	**-**	**-**
	1	4.69	5.06	7.57	**5.77**	27.14	**85.7**	**85.7**
	2	4.58	5.66	5.08	**5.11**	10.58	**75.8**	**87.1**
	3	4.54	4.61	4.32	**4.49**	3.37	**66.7**	**87.4**
	4	4.24	4.73	5.22	**4.73**	10.36	**70.2**	**91.5**
	5	6.08	5.57	4.75	**5.47**	12.27	**81.1**	**95.9**
LifeNet®	0	10.53	10.74	10.56	**10.61**	1.07	**-**	**-**
	1	7.16	8.15	7.68	**7.66**	6.46	**72.2**	**72.2**
	2	7.18	7.17	6.97	**7.11**	1.67	**67.0**	**81.8**
	3	6.61	6.80	6.46	**6.62**	2.57	**62.4**	**85.5**
	4	6.48	5.96	6.79	**6.41**	6.54	**60.4**	**88.2**
	5	5.18	5.37	5.47	**5.34**	2.76	**50.3**	**87.2**
Olyset®	0	21.65	20.21	19.59	**20.48**	5.16	**-**	**-**
	1	19.96	19.80	19.79	**19.85**	0.48	**96.9**	**96.9**
	2	21.74	21.50	19.96	**21.07**	4.58	**102.8**	**101.4**
	3	19.78	20.22	19.91	**19.97**	1.13	**97.5**	**99.2**
	4	19.12	21.37	19.72	**20.07**	5.81	**98.0**	**99.5**
	5	19.99	20.21	19.41	**19.87**	2.08	**97.0**	**99.4**
PermaNet® 2.0	0	1.15	1.15	1.20	**1.17**	2.47	**-**	**-**
	1	1.03	1.12	0.98	**1.04**	6.80	**89.4**	**89.4**
	2	0.98	0.96	1.16	**1.03**	10.66	**88.6**	**94.1**
	3	0.90	0.90	1.05	**0.95**	9.12	**81.4**	**93.4**
	4	0.83	0.89	0.96	**0.89**	7.28	**76.6**	**93.5**
	5	0.83	0.85	0.93	**0.87**	6.08	**74.6**	**94.3**

(*) Each result is the mean of 2 chromatographic injections (duplicate injections).

(**) $w = 100 \times \sqrt[n]{(t_n/t_0)}$

where:

t_n = total active ingredient content (in g/kg) after n washing cycles. This value is calculated by averaging the total active ingredient content of the 3 pieces.

t_0 = total active ingredient content (in g/kg) before washing (no washing). This value is calculated by averaging the total active ingredient content of the 3 pieces.

w = wash resistance index, expressed as percentage.

6.3 Laboratory 3

LN	Wash	Active ingredient content (g/kg)					a.i. wash resistance	Average a.i. wash resistance
		A (*)	B (*)	C (*)	Mean	RSD (%)	(% of wash 0)	(% at each wash) (**)
Interceptor®	0	6.35	6.45	6.37	**6.39**	0.83	**-**	**-**
	1	4.91	7.50	5.29	**5.90**	23.71	**92.3**	**92.3**
	2	5.34	5.00	4.93	**5.09**	4.31	**79.7**	**89.3**
	3	5.60	5.14	4.59	**5.11**	9.90	**80.0**	**92.8**
	4	6.54	4.42	5.37	**5.44**	19.51	**85.2**	**96.1**
	5	6.21	4.46	5.81	**5.49**	16.69	**86.0**	**97.0**
LifeNet®	0	9.98	10.44	10.22	**10.21**	2.23	**-**	**-**
	1	8.07	7.92	8.13	**8.04**	1.36	**78.7**	**78.7**
	2	7.24	7.17	7.42	**7.27**	1.78	**71.2**	**84.4**
	3	7.35	7.00	7.12	**7.15**	2.47	**70.1**	**88.8**
	4	7.15	7.24	6.75	**7.04**	3.65	**69.0**	**91.1**
	5	6.47	6.21	6.96	**6.55**	5.82	**64.1**	**91.5**
Olyset®	0	21.94	20.63	19.20	**20.59**	6.67	**-**	**-**
	1	20.77	20.58	20.11	**20.49**	1.65	**99.5**	**99.5**
	2	19.34	19.42	22.73	**20.50**	9.44	**99.6**	**99.8**
	3	19.93	18.87	18.76	**19.19**	3.36	**93.2**	**97.7**
	4	18.39	19.08	20.36	**19.27**	5.18	**93.6**	**98.4**
	5	19.49	19.05	18.90	**19.14**	1.61	**93.0**	**98.6**
PermaNet® 2.0	0	1.09	1.15	1.09	**1.11**	3.09	**-**	**-**
	1	1.23	1.10	0.98	**1.10**	11.02	**99.6**	**99.6**
	2	0.93	0.96	1.16	**1.02**	11.99	**92.0**	**95.9**
	3	0.90	0.90	0.93	**0.91**	1.89	**82.2**	**93.7**
	4	0.98	0.97	0.93	**0.96**	2.57	**86.9**	**96.6**
	5	1.56	0.86	0.84	**1.09**	37.71	**98.4**	**99.7**

(*) Each result is the mean of 2 chromatographic injections (duplicate injections).

(**) $w = 100 \times \sqrt[n]{(t_n/t_0)}$

where:

t_n = total active ingredient content (in g/kg) after n washing cycles. This value is calculated by averaging the total active ingredient content of the 3 pieces.

t_0 = total active ingredient content (in g/kg) before washing (no washing). This value is calculated by averaging the total active ingredient content of the 3 pieces.

w = wash resistance index, expressed as percentage.

6.4 Laboratory 4

LN	Wash	Active ingredient content (g/kg)					a.i. wash resistance	Average a.i. wash resistance
		A (*)	B (*)	C (*)	Mean	RSD (%)	(% of wash 0)	(% at each wash) (**)
Interceptor®	0	6.30 7.79	6.51 6.88	6.22 6.10	**6.63**	9.49	**100.00**	**-**
	1	6.24 6.19	6.24 5.96	~~8.66~~ 5.80	**6.09**	3.24	**91.75**	**91.75**
	2	5.78 7.64	5.90 7.71	6.32 6.59	**6.66**	12.63	**100.35**	**100.18**
	3	6.44 5.89	5.55 5.84	6.21 ~~2.23~~	**5.99**	5.77	**90.24**	**96.64**
	4	6.85 5.69	5.46 6.12	6.36 5.59	**6.01**	8.87	**90.63**	**97.57**
	5	7.26 5.19	6.08 5.53	7.12 3.36	**5.76**	24.98	**86.78**	**97.20**
LifeNet®	0	10.41	10.25	10.30	**10.32**	0.79	**100.00**	**-**
	1	9.79	9.78	9.86	**9.81**	0.44	**95.06**	**95.06**
	2	9.54	9.56	9.64	**9.58**	0.55	**92.83**	**96.35**
	3	9.14	9.23	9.25	**9.21**	0.64	**89.21**	**96.27**
	4	8.96	8.90	9.17	**9.01**	1.57	**87.31**	**96.66**
	5	9.02	8.93	8.93	**8.96**	0.58	**86.82**	**97.21**
Olyset® Net	0	20.04	19.99	20.18	**20.07**	0.49	**100.00**	**-**
	1	19.96	19.78	20.19	**19.98**	1.03	**99.53**	**99.53**
	2	19.77	19.98	20.06	**19.94**	0.75	**99.34**	**99.67**
	3	19.35	20.10	19.94	**19.80**	2.00	**98.64**	**99.54**
	4	19.85	19.60	19.75	**19.73**	0.64	**98.32**	**99.58**
	5	19.81	19.44	19.89	**19.71**	1.22	**98.22**	**99.64**
PermaNet® 2.0 (Original sample)	0	1.21	1.15	1.08	**1.15**	5.67	**100.00**	**-**
	1	1.06	1.27	1.06	**1.13**	10.73	**98.55**	**98.55**
	2	1.06	1.06	1.01	**1.04**	2.77	**90.99**	**95.38**
	3	1.02	1.04	1.01	**1.02**	1.49	**89.24**	**96.28**
	4	1.01	0.99	1.05	**1.02**	3.00	**88.66**	**97.04**
	5	1.78	1.11	1.84	**1.58**	25.70	**137.50**	**106.58**
PermaNet® 2.0 (Second sample)	0	1.27	1.02	1.31	**1.20**	13.10	**100.00**	**-**
	1	1.31	1.25	1.27	**1.28**	2.39	**106.39**	**106.39**
	2	1.12	0.96	0.95	**1.01**	9.44	**84.17**	**91.74**
	3	1.06	0.97	1.21	**1.08**	11.23	**90.00**	**96.55**
	4	1.06	0.94	1.24	**1.08**	13.98	**90.00**	**97.40**
	5	1.04	1.21	1.08	**1.11**	8.01	**92.50**	**98.45**

(*) Each result is the mean of 2 chromatographic injections (duplicate injections).

(**) $w = 100 \times \sqrt[n]{(t_n/t_0)}$

where:

t_n = total active ingredient content (in g/kg) after n washing cycles. This value is calculated by averaging the total active ingredient content of the 3 pieces.

t_0 = total active ingredient content (in g/kg) before washing (no washing). This value is calculated by averaging the total active ingredient content of the 3 pieces.

w = wash resistance index, expressed as percentage.

6.5 Laboratory 5

LN	Wash	Active ingredient content (g/kg)					a.i. wash resistance	Average a.i. wash resistance
		A (*)	B (*)	C (*)	Mean	RSD (%)	(% of wash 0)	(% at each wash) (**)
Interceptor®	0	6.43	6.58	6.47	**6.49**	1.16	**-**	**-**
	1	5.44	5.68	5.58	**5.57**	2.18	**85.8**	**85.8**
	2	5.27	5.07	5.07	**5.14**	2.34	**79.1**	**88.9**
	3	4.63	4.50	4.68	**4.60**	2.06	**70.9**	**89.2**
	4	4.40	4.49	4.44	**4.44**	1.10	**68.4**	**90.9**
	5	4.32	4.51	4.39	**4.41**	2.19	**67.9**	**92.6**
LifeNet®	0	8.58	8.66	8.68	**8.64**	0.65	**-**	**-**
	1	7.45	7.53	7.54	**7.51**	0.60	**86.9**	**86.9**
	2	6.95	7.25	7.05	**7.08**	2.21	**82.0**	**90.5**
	3	6.50	6.49	6.38	**6.45**	1.03	**74.7**	**90.7**
	4	6.04	5.96	6.11	**6.04**	1.20	**69.8**	**91.4**
	5	5.83	5.70	5.77	**5.77**	1.20	**66.7**	**92.2**
Olyset®	0	20.12	20.14	20.31	**20.19**	0.50	**-**	**-**
	1	18.97	19.35	19.26	**19.19**	1.03	**95.1**	**95.1**
	2	19.17	19.05	18.95	**19.06**	0.57	**94.4**	**97.2**
	3	18.77	18.92	19.23	**18.98**	1.22	**94.0**	**98.0**
	4	18.48	18.85	18.71	**18.68**	0.99	**92.5**	**98.1**
	5	18.79	19.13	19.04	**18.99**	0.93	**94.0**	**98.8**
PermaNet® 2.0	0	1.50	1.58	1.53	**1.54**	2.58	**-**	**-**
	1	1.27	1.26	1.29	**1.27**	1.13	**82.7**	**82.7**
	2	1.17	1.13	1.17	**1.16**	1.88	**75.4**	**86.8**
	3	1.10	1.14	1.09	**1.11**	2.61	**72.3**	**89.8**
	4	1.22	1.02	1.16	**1.13**	9.27	**73.8**	**92.7**
	5	1.07	1.09	1.11	**1.09**	1.95	**70.9**	**93.4**

(*) Each result is the mean of 2 chromatographic injections (duplicate injections).

(**) $w = 100 \times \sqrt[n]{(t_n/t_0)}$

where:

t_n = total active ingredient content (in g/kg) after n washing cycles. This value is calculated by averaging the total active ingredient content of the 3 pieces.

t_0 = total active ingredient content (in g/kg) before washing (no washing). This value is calculated by averaging the total active ingredient content of the 3 pieces.

w = wash resistance index, expressed as percentage.

7 Evaluation and discussion

5 labs participated in the small scale collaborative trial on the wash resistance index of LN and received the samples from the coordinator. All the participating laboratories sent a complete data set of results back to the organiser. The data from each laboratory were reviewed and the calculations (mean and RSD of the active ingredient content, a.i. wash resistance and average a.i. wash resistance were checked to determine if there were any problems with the procedure and methods used which may affect the analysis results. Lab 4 provided 2 sets of results for PermaNet 2.0. The second set was retained in the statistical evaluation because results from this second set were more consistent.

No significant changes from the washing method were reported by laboratories. Laboratories reported some changes from the analytical methods used for determination of active ingredient content in the unwashed and washed samples. These deviations are not expected to affect the analyses results significantly.

The active substance content (mean of the 3 net pieces) in the 4 unwashed LNs and the 4 LNs washed 1, 2, 3, 4 and 5 times is presented in Table 1. The wash curves (active ingredient content in function of the number of washes) are presented in Figures 1, 2, 3 and 4, respectively for Interceptor®, LifeNet®, Olyset® and PermaNet® 2.0. The best equation between the total active ingredient content and the number of washes is obtained using an exponential regression curve. This corresponds to a free-migration stage behaviour (see report of the eleventh WHOPES Working Group Meeting, WHO/HQ, Geneva, 10-13 December 2007, WHO/HTM/NTD/WHOPES/2008.1). A proportion of the total active ingredient content of small molecules (including a proportion of active ingredient) is removed from the surface at each wash. After equilibration, the same proportion (but a smaller quantity) is removed by the next wash, so the decline in total content is non-linear.

The inter-laboratory relative standard deviation (RSD_R) of the active substance content ranges from 3.2% to 12.9% for Interceptor®, from 13.0% to 21.0% for LifeNet®, from 1.3% to 3.7% for Olyset® and from 7.1% to 13.3% for PermaNet® 2.0. This variation is already observed in the unwashed nets and is not correlated with the number of washes, suggesting an acceptable repeatability of the washing procedure. This variation reflects therefore the variation of the active ingredient content among the net and includes also the variation of the analytical method for active ingredient content. This variation is higher for multifilament polyester coated nets (Interceptor® and PermaNet® 2.0) and multifilament polypropylene incorporated net (LifeNet®) than for monofilaments polyethylene incorporated nets (Olyset). The higher variation observed for LifeNet® could also be due to the analytical method for active ingredient content which includes an additional evaporation step of the extraction solvent before the chromatographic HPLC injections.

Table 1 : Active substance content (mean of the 3 net pieces) in the 4 unwashed LNs and the 4 LNs washed 1, 2, 3, 4 and 5 times.

LN	Wash	Active substance content (g/kg)					Mean	RSD_R
		Labo 1	Labo 2	Labo 3	Labo 4	Labo 5	(g/kg)	(%)
Interceptor®	0	6.01	6.74	6.39	6.63	6.49	**6.5**	**4.3**
	1	5.82	5.77	5.90	6.09	5.57	**5.8**	**3.2**
	2	5.54	5.11	5.09	6.66	5.14	**5.5**	**12.2**
	3	5.46	4.49	5.11	5.99	4.60	**5.1**	**12.0**
	4	5.81	4.73	5.44	6.01	4.44	**5.3**	**12.9**
	5	5.43	5.47	5.49	5.76	4.41	**5.3**	**9.8**
LifeNet®	0	12.45	10.61	10.21	10.32	8.64	**10.4**	**13.0**
	1	10.13	7.66	8.04	9.81	7.51	**8.6**	**14.4**
	2	9.03	7.11	7.27	9.58	7.08	**8.0**	**14.9**
	3	8.84	6.62	7.15	9.21	6.45	**7.7**	**16.7**
	4	7.79	6.41	7.04	9.01	6.04	**7.3**	**16.3**
	5	6.90	5.34	6.55	8.96	5.77	**6.7**	**21.0**
Olyset®	0	20.00	20.48	20.59	20.07	20.19	**20.3**	**1.3**
	1	19.79	19.85	20.49	19.98	19.19	**19.9**	**2.3**
	2	19.88	21.07	20.50	19.94	19.06	**20.1**	**3.7**
	3	19.64	19.97	19.19	19.80	18.98	**19.5**	**2.1**
	4	19.58	20.07	19.27	19.73	18.68	**19.5**	**2.7**
	5	19.52	19.87	19.14	19.71	18.99	**19.4**	**1.9**
PermaNet® 2.0	0	1.29	1.17	1.11	1.20	1.54	**1.3**	**13.3**
	1	1.14	1.04	1.10	1.28	1.27	**1.2**	**8.8**
	2	1.16	1.03	1.02	1.01	1.16	**1.1**	**7.1**
	3	1.11	0.95	0.91	1.08	1.11	**1.0**	**9.2**
	4	1.03	0.89	0.96	1.08	1.13	**1.0**	**9.3**
	5	1.02	0.87	1.09	1.11	1.09	**1.0**	**9.6**

Figure 1 : Wash curves for Interceptor®

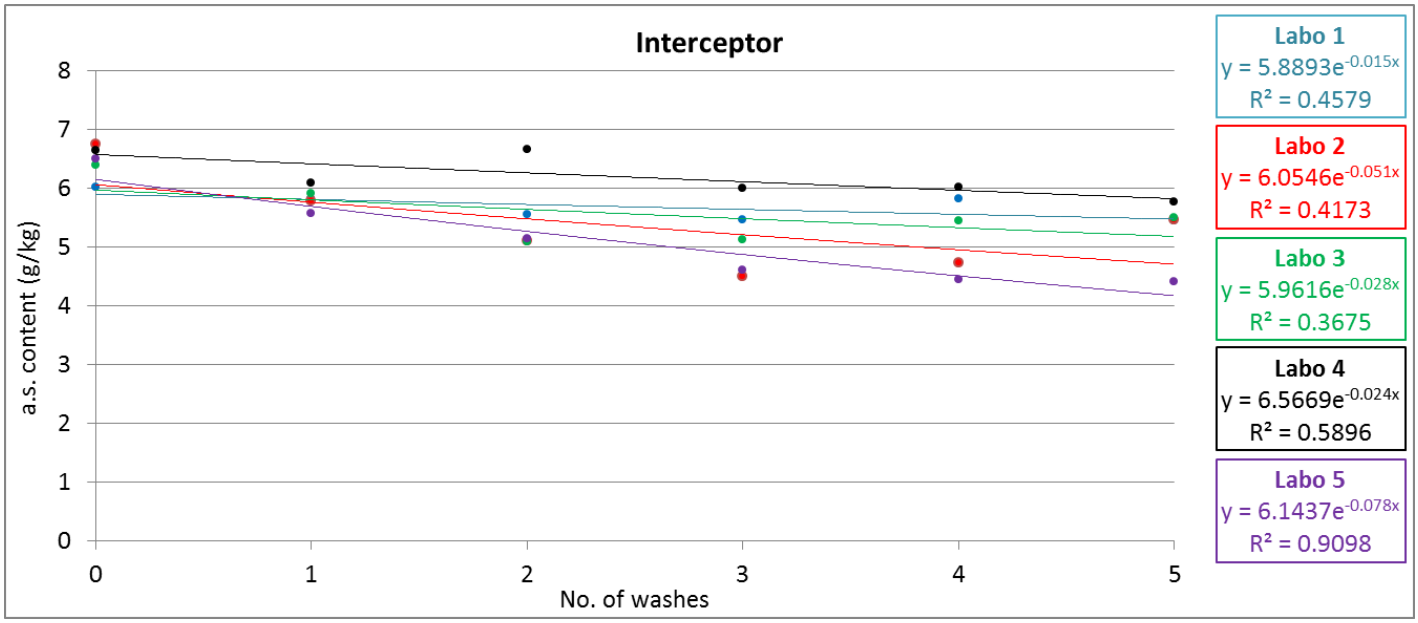


Figure 2 : Wash curves for Lifenet®

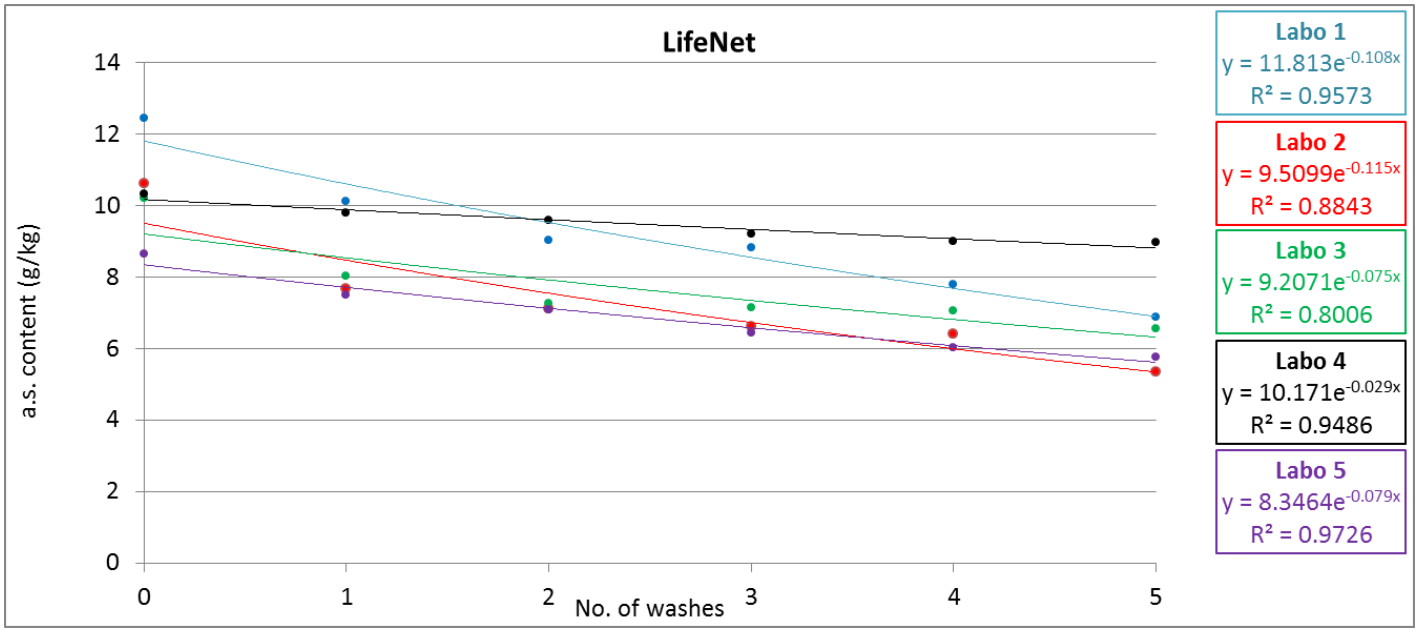


Figure 3 : Wash curves for Olyset®

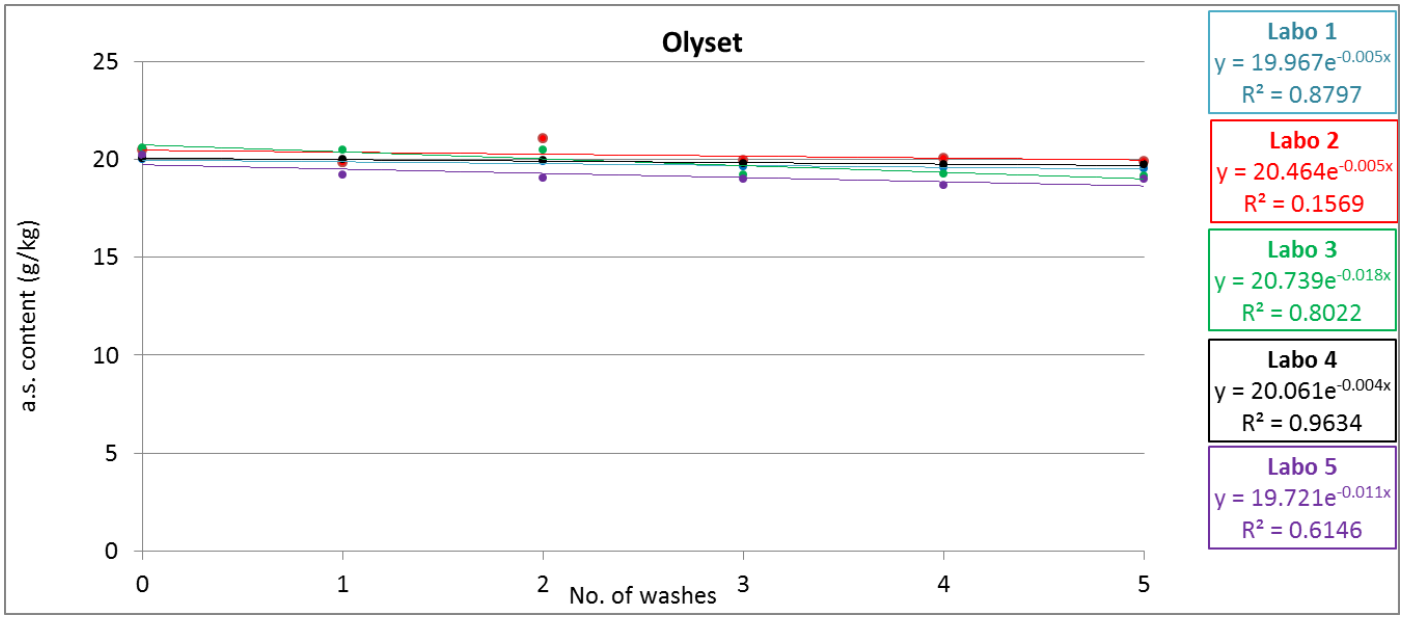
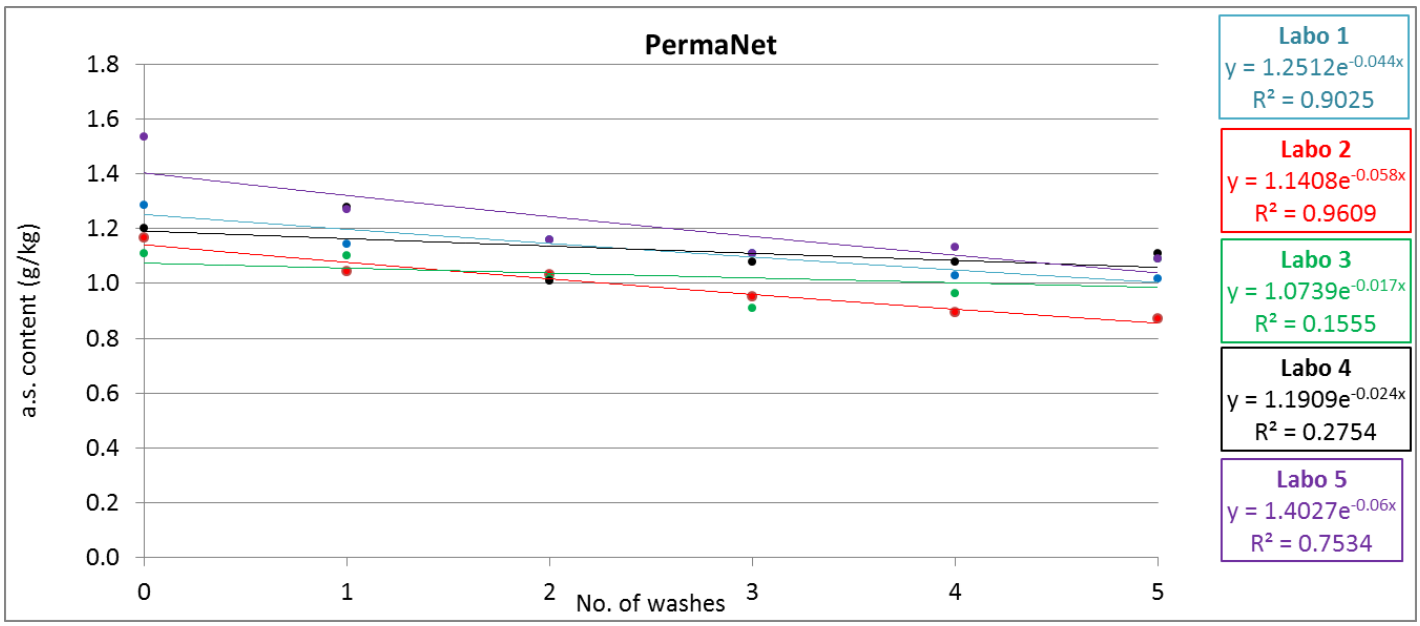


Figure 4 : Wash curves for PermaNet® 2.0

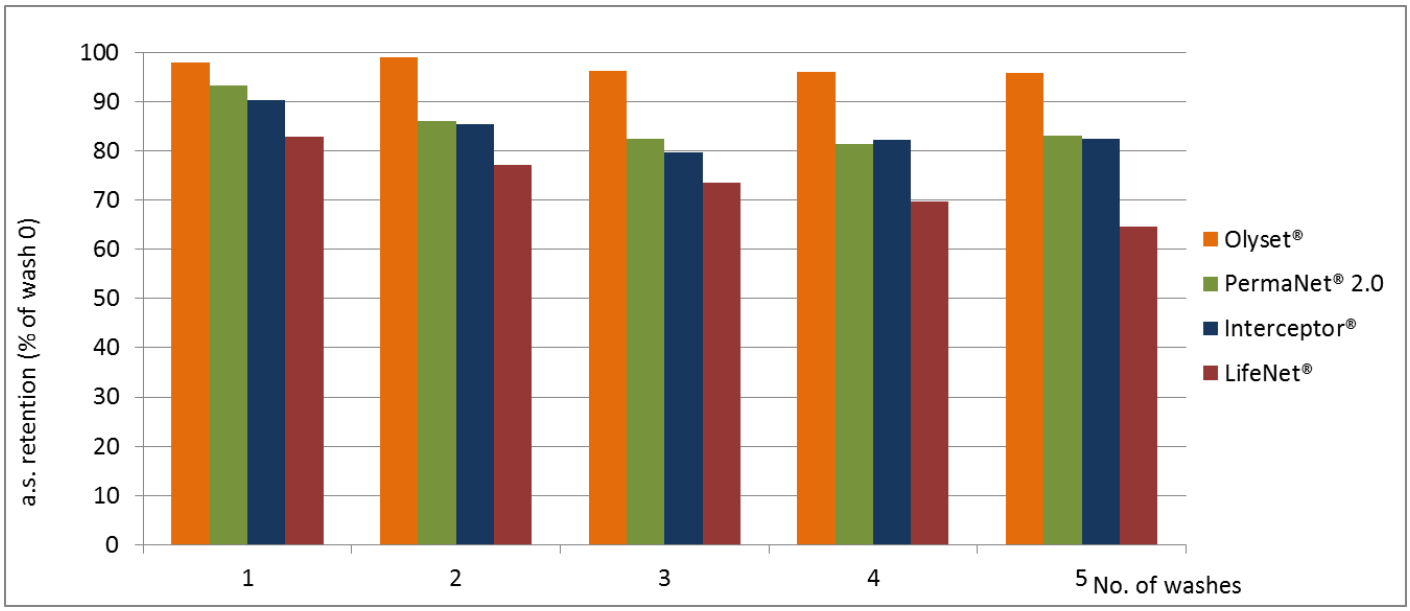


The active substance wash resistance (% of wash 0 - mean of the 3 net pieces) in the 4 LNs washed 1, 2, 3, 4 and 5 times is presented in Table 2 and Figure 5.

Table 2 : Active substance wash resistance (% of wash 0) for the 4 LNs washed 1, 2, 3, 4 and 5 times.

LN	Wash	Active substance wash resistance (% of wash 0)					Mean	RSD_R
		Labo 1	Labo 2	Labo 3	Labo 4	Labo 5	(%)	(%)
Interceptor®	0	100.0	100.0	100.0	100.0	100.0	**100.0**	**-**
	1	96.8	85.7	92.3	91.7	85.8	**90.5**	**5.2**
	2	92.2	75.8	79.7	100.4	79.1	**85.4**	**12.2**
	3	90.8	66.7	80.0	90.2	70.9	**79.7**	**13.8**
	4	96.7	70.2	85.2	90.6	68.4	**82.2**	**15.2**
	5	90.4	81.1	86.0	86.8	67.9	**82.4**	**10.6**
LifeNet®	0	100.0	100.0	100.0	100.0	100.0	**100.0**	**-**
	1	81.4	72.2	78.7	95.1	86.9	**82.8**	**10.4**
	2	72.5	67.0	71.2	92.8	82.0	**77.1**	**13.4**
	3	71.0	62.4	70.1	89.2	74.7	**73.5**	**13.4**
	4	62.6	60.4	69.0	87.3	69.8	**69.8**	**15.1**
	5	55.4	50.3	64.1	86.8	66.7	**64.7**	**21.7**
Olyset®	0	100.0	100.0	100.0	100.0	100.0	**100.0**	**-**
	1	99.0	96.9	99.5	99.5	95.1	**98.0**	**2.0**
	2	99.4	102.8	99.6	99.3	94.4	**99.1**	**3.1**
	3	98.2	97.5	93.2	98.6	94.0	**96.3**	**2.6**
	4	97.9	98.0	93.6	98.3	92.5	**96.1**	**2.9**
	5	97.6	97.0	93.0	98.2	94.0	**96.0**	**2.4**
PermaNet® 2.0	0	100.0	100.0	100.0	100.0	100.0	**100.0**	**-**
	1	88.9	89.4	99.6	106.4	82.7	**93.4**	**10.1**
	2	90.2	88.6	92.0	84.2	75.4	**86.1**	**7.7**
	3	86.3	81.4	82.2	90.0	72.3	**82.4**	**8.0**
	4	80.1	76.6	86.9	90.0	73.8	**81.5**	**8.4**
	5	78.9	74.6	98.4	92.5	70.9	**83.1**	**14.3**

Figure 5 : Active substance wash resistance (% of wash 0) for the 4 LNs washed 1, 2, 3, 4 and 5 times.



The average wash resistance (% at each wash - mean of the 3 net pieces) in the 4 LNs washed 1, 2, 3, 4 and 5 times is presented in Table 3. Figures 6, 7 and 8 present the average wash resistance after 3, 4 and 5 washes respectively.

The inter-laboratory relative standard deviation (RSD_R) of the average wash resistance index after 4 washes (as recommended in the CIPAC method) is 3.8% for Interceptor®, 3.6% for LifeNet, 0.7% for Olyset® and 2.1% for PermaNet® 2.0 and shows the acceptable reproducibility of the washing method. The inter-laboratory relative standard deviation (RSD_R) of the average wash resistance index after 3 and 5 washes is very close to the value obtained after 4 washes and confirm the accuracy and reproducibility of the washing method.

Table 3 : Average active substance wash resistance (% of wash 0) for the 4 LNs washed 1, 2, 3, 4 and 5 times.

LN	Wash	Average active substance wash resistance (% at each wash)					Mean	RSD_R
		Labo 1	Labo 2	Labo 3	Labo 4	Labo 5	(%)	(%)
Interceptor®	0	100.0	100.0	100.0	100.0	100.0	**100.0**	**-**
	1	96.8	85.7	92.3	91.7	85.8	**90.5**	**5.2**
	2	96.0	87.1	89.3	100.2	88.9	**92.3**	**6.0**
	3	96.9	87.4	92.8	96.6	89.2	**92.6**	**4.6**
	4	99.2	91.5	96.1	97.6	90.9	**95.1**	**3.8**
	5	98.0	95.9	97.0	97.2	92.6	**96.1**	**2.2**
LifeNet®	0	100.0	100.0	100.0	100.0	100.0	**100.0**	**-**
	1	81.4	72.2	78.7	95.1	86.9	**82.8**	**10.4**
	2	85.2	81.8	84.4	96.3	90.5	**87.7**	**6.6**
	3	89.2	85.5	88.8	96.3	90.7	**90.1**	**4.4**
	4	88.9	88.2	91.1	96.7	91.4	**91.3**	**3.6**
	5	88.9	87.2	91.5	97.2	92.2	**91.4**	**4.2**
Olyset®	0	100.0	100.0	100.0	100.0	100.0	**100.0**	**-**
	1	99.0	96.9	99.5	99.5	95.1	**98.0**	**2.0**
	2	99.7	101.4	99.8	99.7	97.2	**99.5**	**1.5**
	3	99.4	99.2	97.7	99.5	98.0	**98.7**	**0.9**
	4	99.5	99.5	98.4	99.6	98.1	**99.0**	**0.7**
	5	99.5	99.4	98.6	99.6	98.8	**99.2**	**0.5**
PermaNet® 2.0	0	100.0	100.0	100.0	100.0	100.0	**100.0**	**-**
	1	88.9	89.4	99.6	106.4	82.7	**93.4**	**10.1**
	2	95.0	94.1	95.9	91.7	86.8	**92.7**	**3.9**
	3	95.2	93.4	93.7	96.5	89.8	**93.7**	**2.7**
	4	94.6	93.5	96.6	97.4	92.7	**95.0**	**2.1**
	5	95.4	94.3	99.7	98.5	93.4	**96.2**	**2.8**

Figure 6 : Average active substance wash resistance (% at each wash) for the 4 LNs washed 3 times.

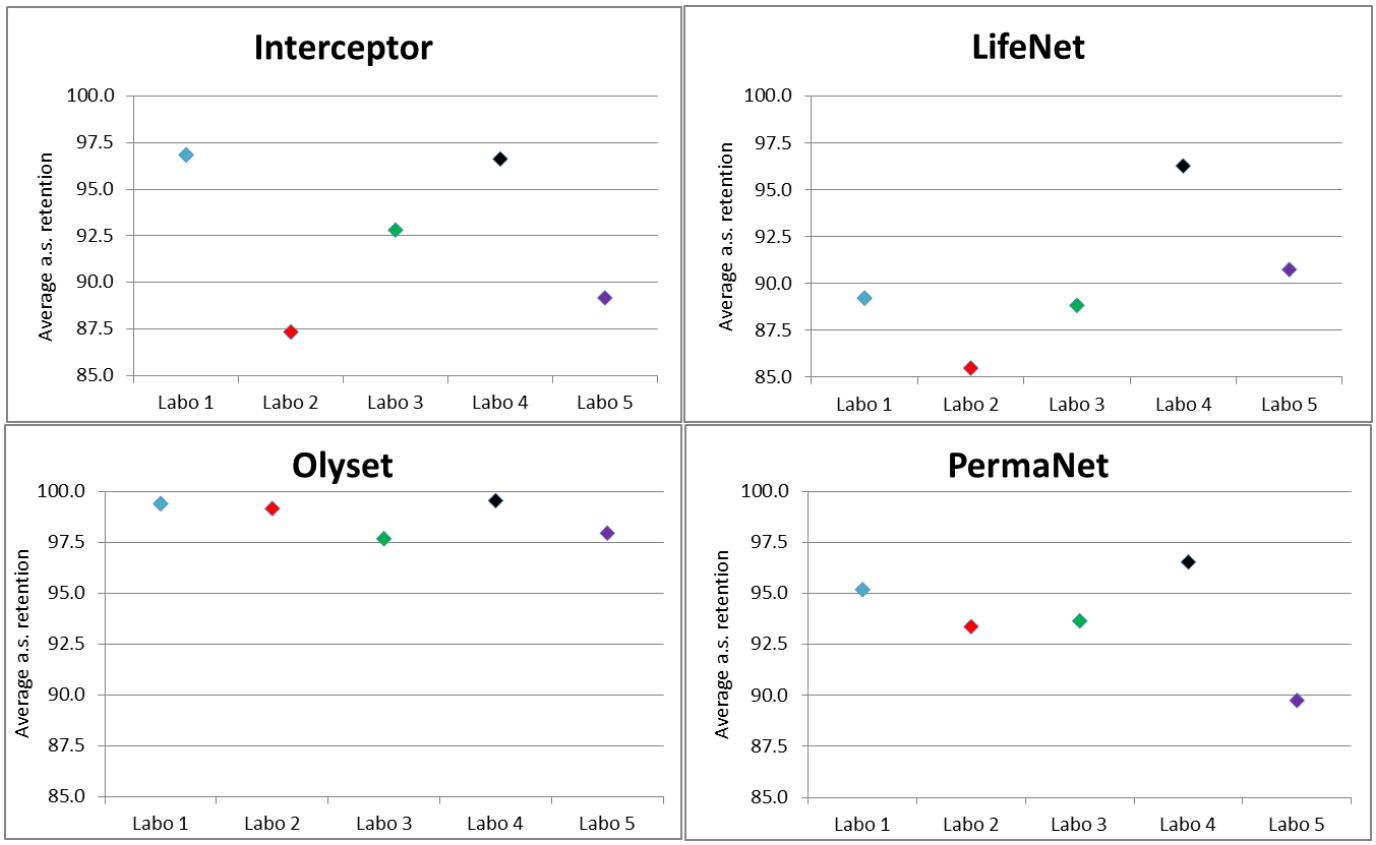


Figure 7 : Average active substance wash resistance (% at each wash) for the 4 LNs washed 4 times.

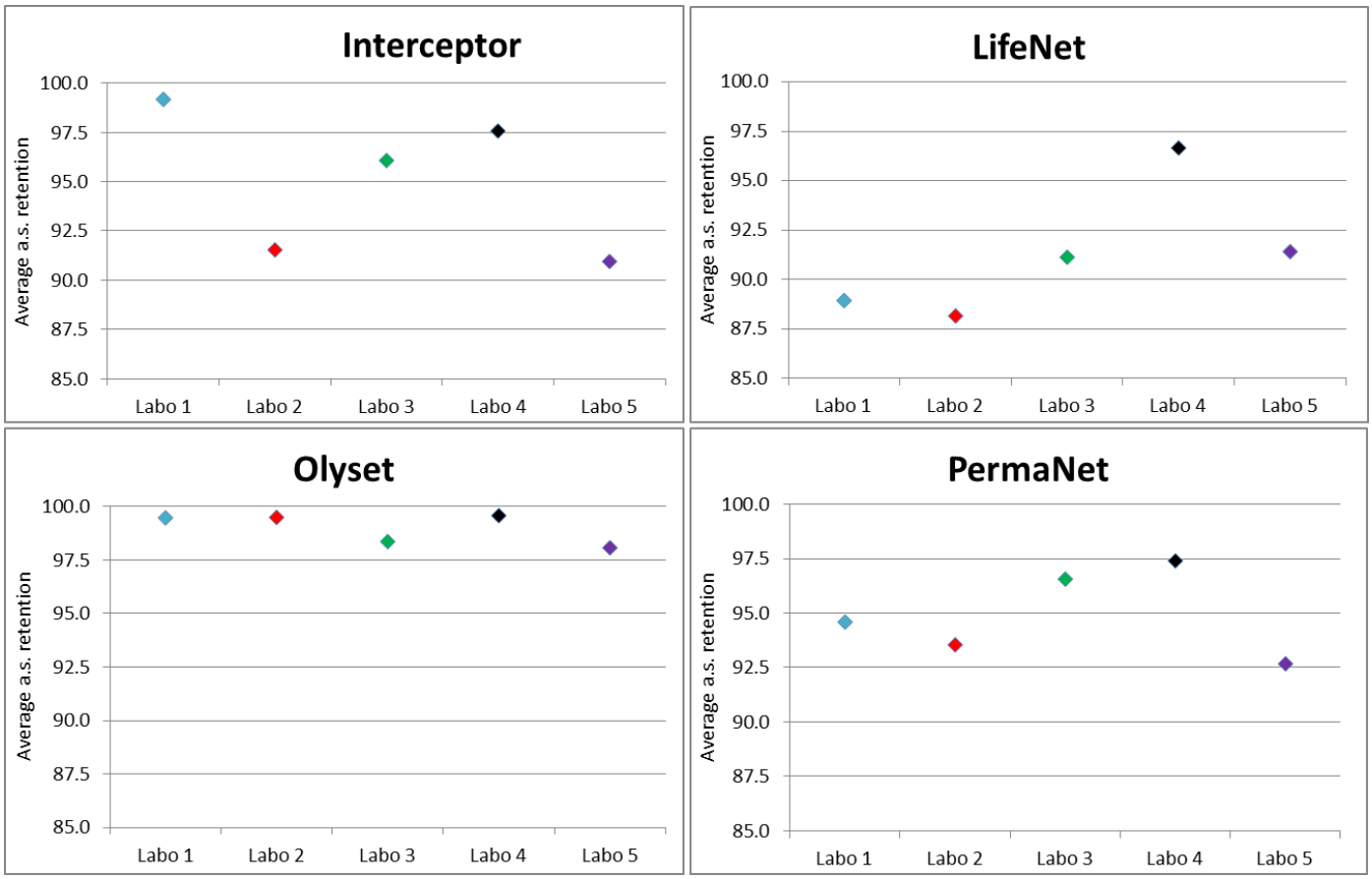
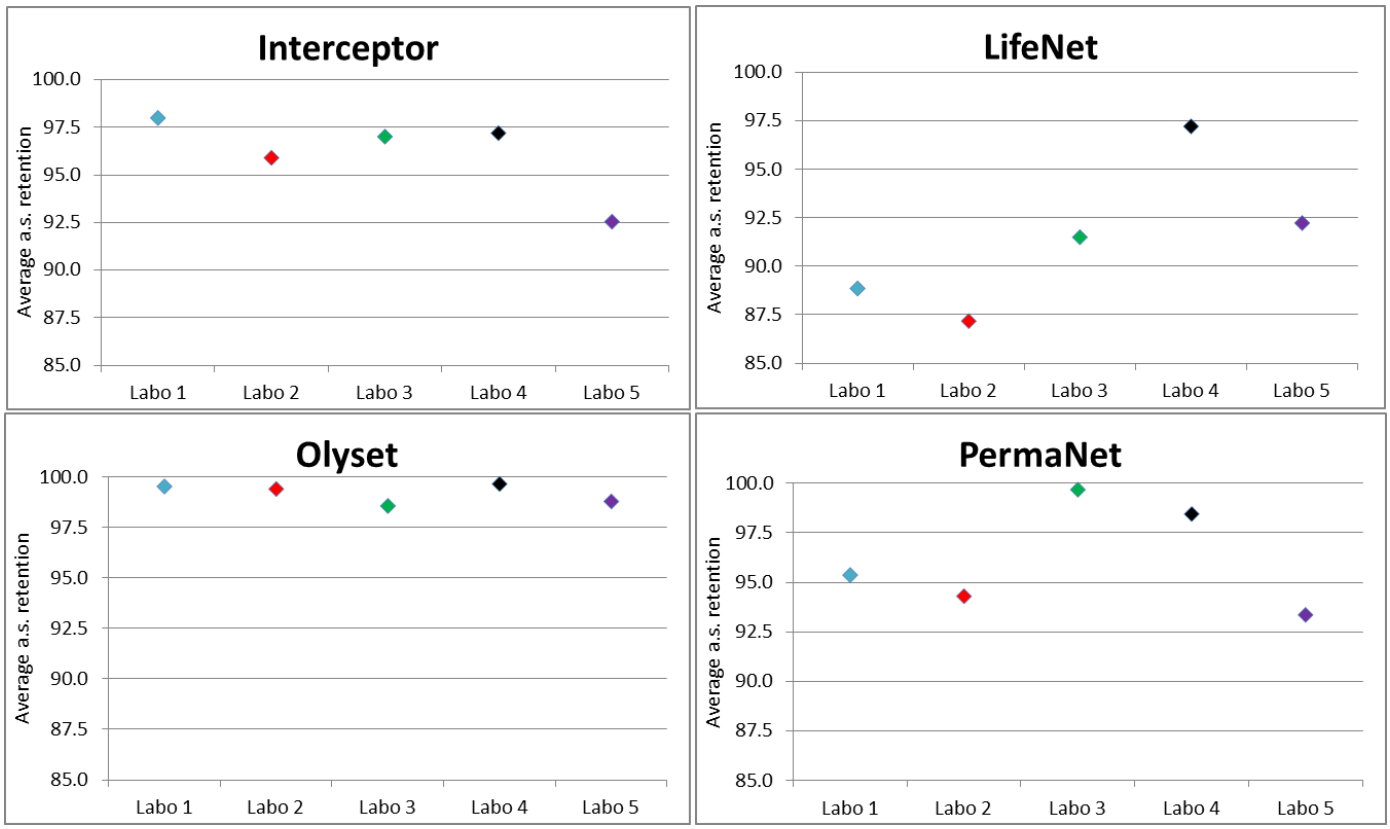


Figure 8 : Average active substance wash resistance (% at each wash) for the 4 LNs washed 5 times.



A comparison of the wash resistance index (lab range and mean) obtained from the 5 laboratories for the 4 LNs with the criteria of the WHO specifications is presented in Table 4. This comparison shows that the WHO specifications should be revised taking into account the inter-laboratory variation of the wash resistance index method, particularly for LifeNet® and Olyset®.

Table 4 : Average wash resistance index for the 4 LNs washed 4 times and WHO specifications.

LN	Average active ingredient retention index at wash 4		WHO specification criteria
	Lab range	Mean	
Interceptor®	90.9-99.2	95.1	**90-98**
LifeNet®	88.2-96.7	91.3	**94-99**
Olyset®	98.1-99.6	99.0	**Release index**
PermaNet® 2.0	92.7-97.4	95.0	**87-97**

8 Summary and conclusions

A Small Scale Collaborative Trial was performed on the CIPAC method for determination of the wash resistance index of long-lasting insecticidal mosquito nets (LNs). This method is a further standardization of the current WHO washing method and involves the standardization of the washing agent, the washing movement, the heating (regeneration) step, and the clarification of the calculation of the wash resistance index. The trial involved the testing of 4 different LNs (Interceptor®, LifeNet®, Olyset® and PermaNte® 2.0) having different physico-chemical properties : different pyrethroids active ingredients (alpha-cypermethrin, deltamethrin), different active ingredients content, different yarn types (monofilament, multifilament), different manufacturing technologies (coated onto polyester, incorporated into polyethylene, incorporated into polypropylene).

The inter-laboratory relative standard deviation (RSD_R) of the average wash resistance index after 4 washes (0.7 - 3.8%) is larger than those of CIPAC methods for active ingredient content and can be explained by the fact that LNs are not as homogeneous than other formulation types and that the washing steps can give rise to larger variations. Nevertheless the variation is acceptable for a such kind of method.

The method is straightforward and easy to carry out. If the washing and rinsing operations are well planned and organized, the method permit to perform the washing and rinsing of 36 net pieces (12 samples in triplicate) in a half day with 2 technicians.

This small scale trial involving 5 laboratories on 4 LNs gave acceptable results for a physico-chemistry method and is considered as an indirect validation of the washing method. We consider this method to be suitable for determination of the wash resistance index of LN and recommend accepting it as a provisional CIPAC method.

We propose to include in the method the minor changes proposed by the laboratories 3, 4 and 5.

- The use of a thermostated oven at 30°C ± 2°C as an option for laboratories not having access to a vibration free water bath.
- In the heating step : “fold the sample carefully once or twice in each direction” instead of “fold the sample carefully once in each direction”.
- In the assay for determination of the total active ingredient content, adding of a footnote specifying that “Unwashed samples and wash samples must be analysed simultaneously to reduce the analytical error”.

9 Acknowledgment

We wish to thank all the laboratories and their staff who participated in this study.