DEVELOPMENT OF A CIPAC WASHING METHOD FOR

LONG-LASTING INSECTICIDAL NETS (LN)

Pre-testing of the shaking movement for washing/rinsing

REPORT

Markus Müller, Olivier Pigeon, Martin Rodler,
Tsunehisa Fujita, Yumiko Kozuki

Pre-testing of the shaking movement for washing/rinsing

1. Sample

- Olyset® : permethrin 20 g/kg long-lasting (incorporated into polyethylene) insecticidal mosquito net (LN).

2. CIPAC washing agent

12 g sodium oleate

+ 8 g polyoxyethylene glycol (25) monostearate

+ 80 mL deionised water

3. Laboratory

- Sumitomo, Japan (Yumiko Kozuki and Tsunehisa Fujita) for the testing of Olyset®.

4. Study procedure

4.1. Sampling for wash resistance index

From each side face net, 24 times 3 pieces of 25 cm × 25 cm were cut with scissors in the length of the net, parallel to the lower border and put into a 1 L screw capped glass bottle for determination of wash resistance index after 0, 1, 2, 3, 4 and 5 washes with CIPAC washing agent at 8 g/L (3 pieces for each wash cycle) (= 24 samples of 3 pieces for each net).

4.2. Calculation of wash resistance index

Modified WHO washing procedure (= future CIPAC washing method)

[3 determinations per sample]

- Washing [1] of 1 individual piece of 25 cm × 25 cm with 500 mL detergent solution.
- Rinsing 2 times with 500 mL deionized water in a similar way to washing process.
- Drying on a line at ambient temperature protected from direct sunlight for 30 minutes and then at 40 °C ± 2 °C in the dark for a time period of 22 ± 2 hours before the next washing.
- After the wash cycles, storage of the piece into an aluminium foil in a freezer at 4 °C (± 3 °C).

[1] The following movements were compared:
  - Horizontal rolling at 60 rpm at 30 °C ± 2 °C for 10 minutes
  - Horizontal rolling at 210 rpm at 30 °C ± 2 °C for 10 minutes
  - Hand shaking at 30 °C ± 2 °C by inverting the 1 litter bottle 10 times
  - Hand shaking at 30 °C ± 2 °C by inverting the 1 litter bottle 30 times

Determination of active ingredient content

After the washing procedure, determination of active ingredient content in each individual piece [1 determination for each individual piece]. Results were expressed as g active ingredient per kg netting material. The mean and RSD of the 3 pieces for each wash cycle were calculated and the wash resistance index was calculated for each wash cycle using the equation for a free migration stage behaviour (see table below).

Methods for active ingredient content

- Olyset® : CIPAC method 331/LN/M/3 (permethrin in incorporated nets), CIPAC Handbook M, page 159.

5. Results of analysis for Olyset®

LN Detergent Movement	Wash	Active ingredient content (g/kg)					Wash resistance index (% of wash 0)	Average wash resistance index (% at each wash) (**)
		A (*)	B (*)	C (*)	Mean	RSD (%)		
Olyset®	0	19.7	19.1	19.6	19.47	1.7		
	1	19.2	19.1	19.5	19.30	1.1	99.1	99.1
CIPAC washing agent	2	19.0	19.2	19.2	19.13	0.8	98.3	99.1
8 g/L	3	19.3	19.4	18.7	19.13	1.8	98.3	99.4
Horizontal rolling	4	19.0	18.5	19.2	18.89	2.1	97.0	99.3
60 rpm	5	19.0	19.1	18.5	18.88	1.7	97.0	99.4
Olyset®	0	19.7	19.1	19.6	19.47	1.7		
	1	19.3	19.5	19.0	19.23	1.4	98.8	98.8
CIPAC washing agent	2	19.3	18.5	19.0	18.94	1.9	97.3	98.6
8 g/L	3	18.7	19.0	19.2	18.94	1.3	97.3	99.1
Horizontal rolling	4	19.1	18.7	18.9	18.92	1.1	97.2	99.3
210 rpm	5	19.0	18.9	18.8	18.91	0.5	97.1	99.4
Olyset®	0	19.7	19.1	19.6	19.47	1.7		
	1	19.2	19.3	19.4	19.28	0.4	99.1	99.1
CIPAC washing agent	2	19.3	19.1	19.4	19.26	1.0	98.9	99.5
8 g/L	3	18.7	19.1	18.9	18.91	0.9	97.2	99.0
Hand shaking	4	19.0	19.0	18.4	18.81	2.0	96.6	99.1
10 times	5	18.7	18.9	18.8	18.80	0.6	96.6	99.3
Olyset®	0	19.9	19.7	19.7	19.77	0.7		
	1	19.9	19.3	19.4	19.55	1.7	98.9	98.9
CIPAC washing agent	2	19.3	19.6	19.4	19.42	0.9	98.2	99.1
8 g/L	3	19.6	19.2	19.4	19.39	1.0	98.1	99.4
Hand shaking	4	19.3	19.6	19.3	19.38	0.8	98.0	99.5
30 times	5	18.6	18.4	18.4	18.46	0.8	93.4	98.6

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

(**) **Free migration stage behaviour**

Average wash resistance index = $\sqrt[n]{(t_n/t_0)}$

where t_n = active ingredient total content after wash n, g/kg

t_0 = active ingredient total content at wash 0 (pre-washing), g/kg

n = number of washes.

Reporting of the referential result [2]

Test procedure

- From each side face net, 6 times 3 pieces of 25 cm × 25 cm were cut with scissors in the length of the net, parallel to the lower border and put into a 1 L screw capped glass bottle for determination of wash resistance index after 0, 1, 2, 3, 4 and 5 washes with Savon de Marseille at 2 g/L.
- Washing of 1 individual piece of 25 cm × 25 cm with 500 mL soap solution in a reciprocal water bath shaker at 155 beats / minute with an amplitude of 15 mm at 30 °C ± 2 °C for 10 minutes.
- Rinsing 2 times with 500 mL deionized water in a reciprocal water bath shaker at 155 beats / minute with an amplitude of 15 mm at 30 °C ± 2 °C for 10 minutes.
- Drying on a line at ambient temperature protected from direct sunlight for 30 minutes and then at 40 °C ± 2 °C in the dark for a time period of 22 ± 2 hours before the next washing.
- After the wash cycles, storage of the piece into an aluminium foil in a freezer at 4 °C (± 3 °C).
- After the washing procedure, determination of active ingredient content in each individual piece [1 determination for each individual piece]. Results were expressed as g active ingredient per kg netting material. The mean and RSD of the 3 pieces for each wash cycle were calculated and the wash resistance index was calculated for each wash cycle using the equation for a free migration stage behaviour.

[2] The result was reported in the CIPAC 4789/R.

LN	Wash	Active ingredient content (g/kg)					Wash resistance index (% of wash 0)	Average wash resistance index (% at each wash)
Detergent		A	B	C	Mean	RSD		
Movement		(*)	(*)	(*)		(%)		(**)
Olyset®	0	19.9	19.7	19.7	**19.77**	0.7		
	1	19.9	19.1	19.5	**19.49**	2.0	**98.6**	**98.6**
Savon de Marseille	2	19.2	19.4	19.5	**19.38**	0.9	**98.0**	**99.0**
2 g/L	3	19.4	19.2	19.5	**19.37**	1.0	**98.0**	**99.3**
Reciprocal	4	19.5	19.1	19.5	**19.37**	1.3	**98.0**	**99.5**
Shaker	5	18.8	18.8	19.0	**18.87**	0.5	**95.4**	**99.1**

(*) Each result is the mean of 2 chromatographic injections (duplicate injections).
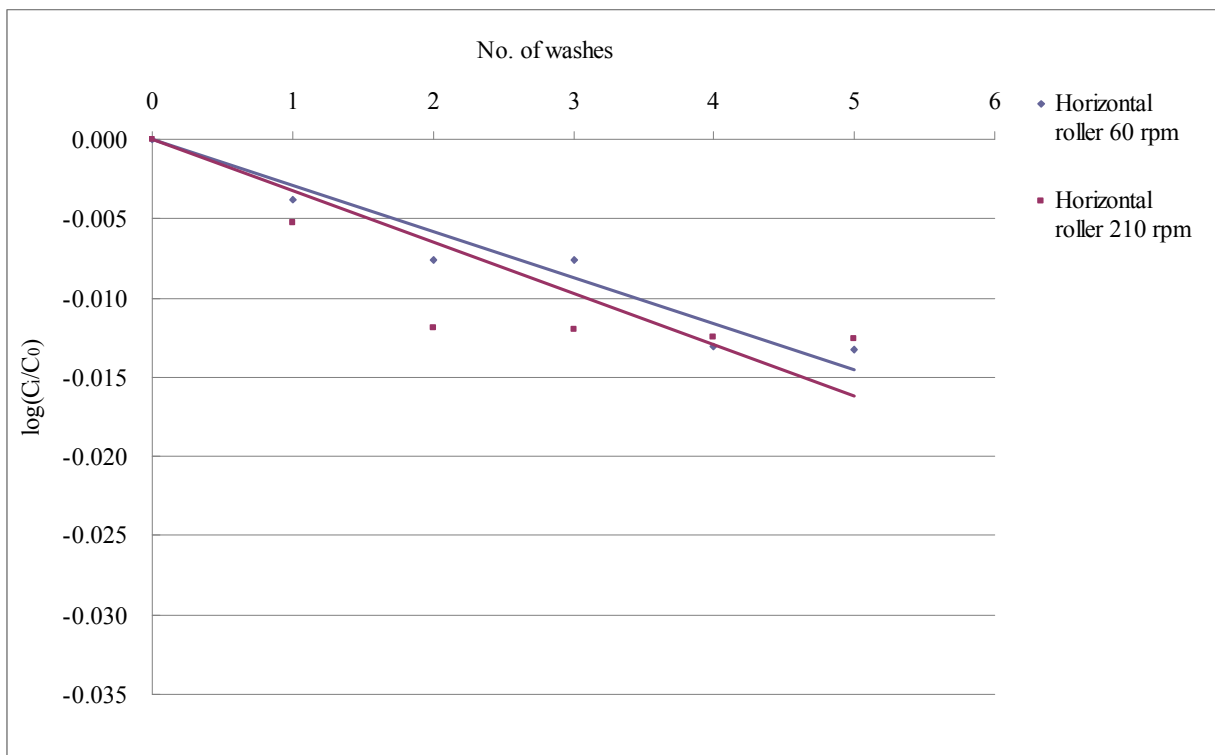
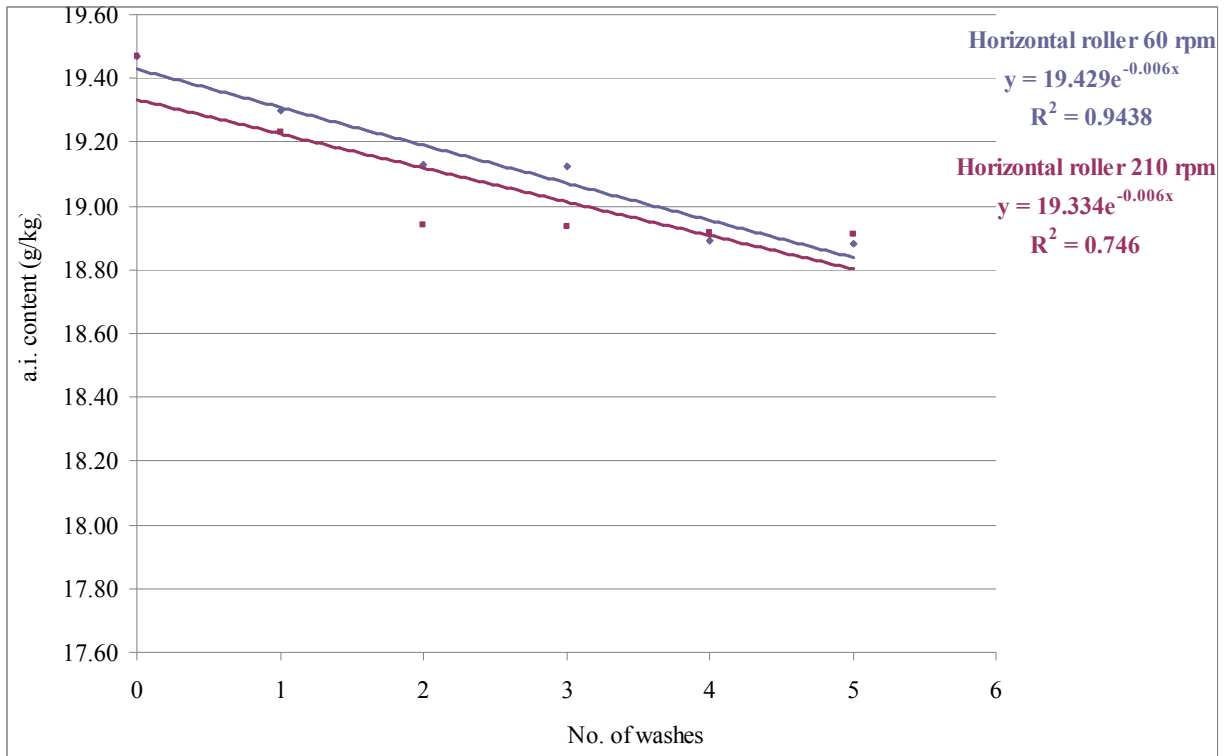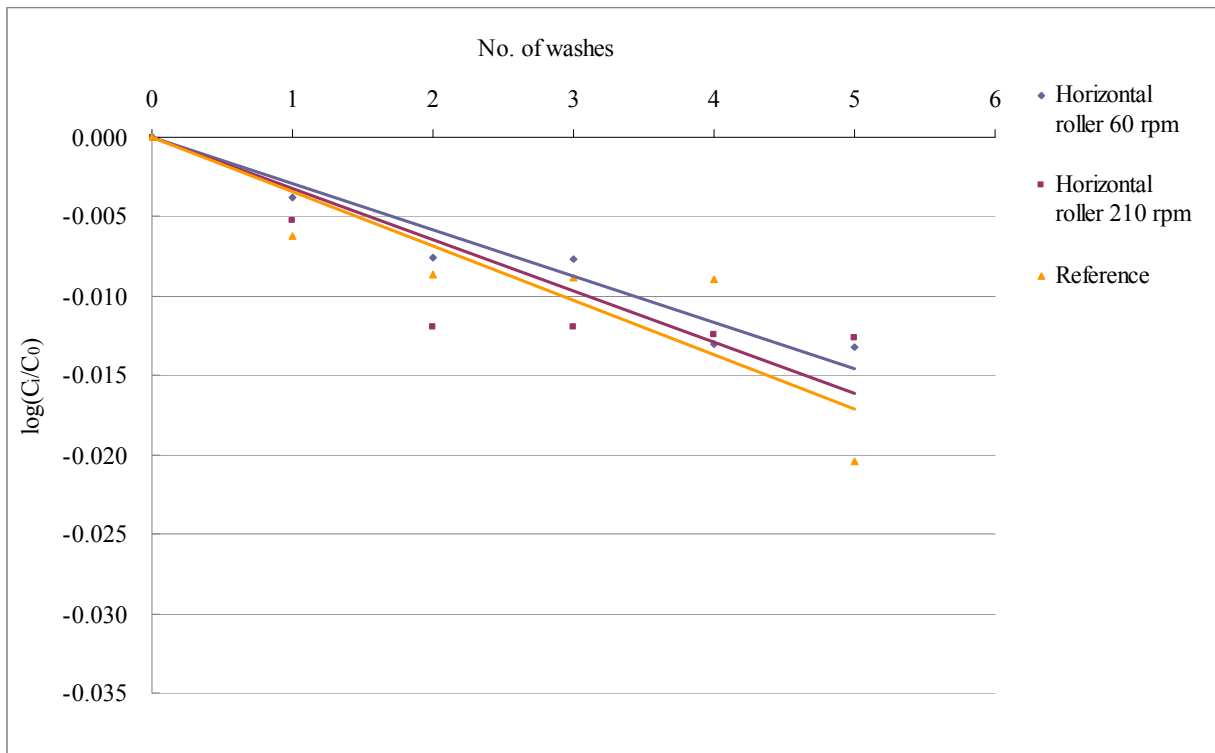(**) **Free migration stage behaviour**

Average wash resistance index = $\sqrt[n]{t_n/t_0}$

where t_n = active ingredient total content after wash n, g/kg

t_0 = active ingredient total content at wash 0 (pre-washing), g/kg

n = number of washes.

Horizontal roller 60 rpm
$y = 19.429e^{-0.006x}$
$R^2 = 0.9438$

Horizontal roller 210 rpm
$y = 19.334e^{-0.006x}$
$R^2 = 0.746$

a.i. content (g/kg)

No. of washes

No. of washes

- Horizontal roller 60 rpm

- Horizontal roller 210 rpm

$\log(C_i/C_0)$

Horizontal roller 60 rpm
$y = 19.429e^{-0.006x}$
$R^2 = 0.9438$

Horizontal roller 210 rpm
$y = 19.334e^{-0.006x}$
$R^2 = 0.746$

Reference
$y = 19.729e^{-0.007x}$
$R^2 = 0.7954$

♦ Horizontal roller 60 rpm

■ Horizontal roller 210 rpm

▲ Reference

Hand shaking 10 times
$y = 19.453e^{-0.008x}$
$R^2 = 0.9238$

Hand shaking 30 times
$y = 19.843e^{-0.011x}$
$R^2 = 0.7004$

Hand shaking 10 times
$y = 19.453e^{-0.008x}$
$R^2 = 0.9238$

Hand shaking 30 times
$y = 19.843e^{-0.011x}$
$R^2 = 0.7004$

Reference
$y = 19.729e^{-0.007x}$
$R^2 = 0.7954$
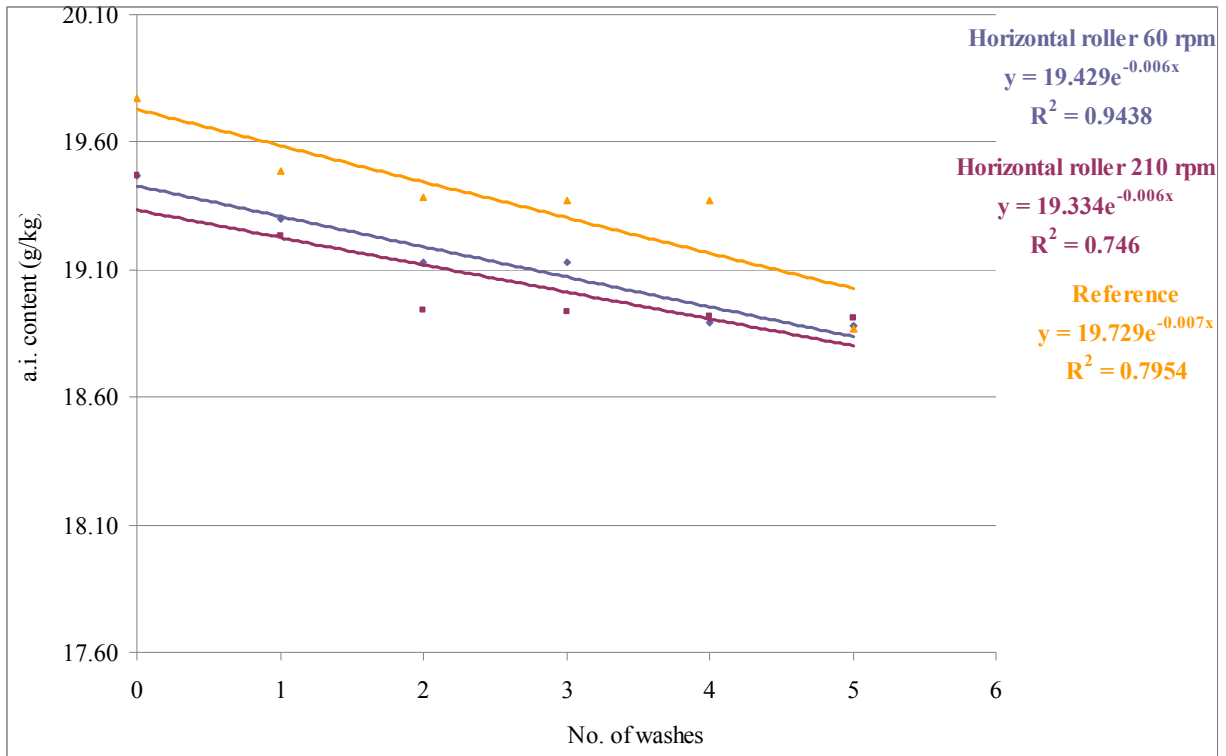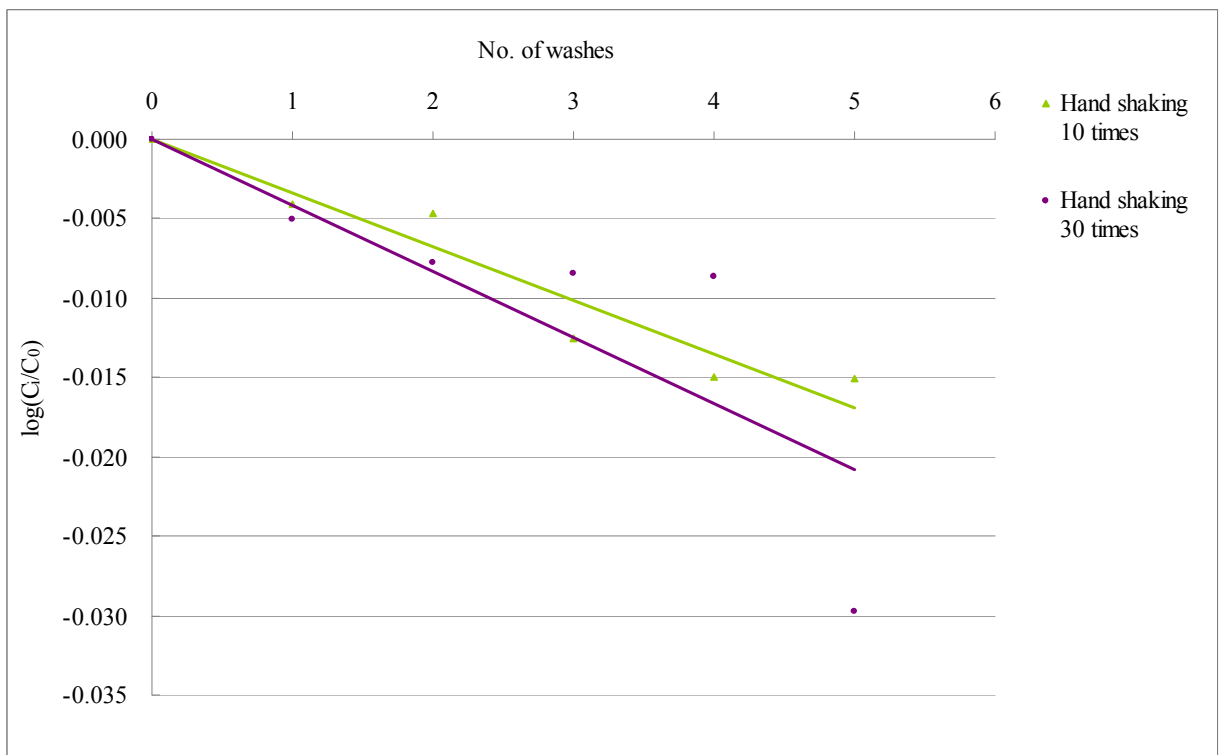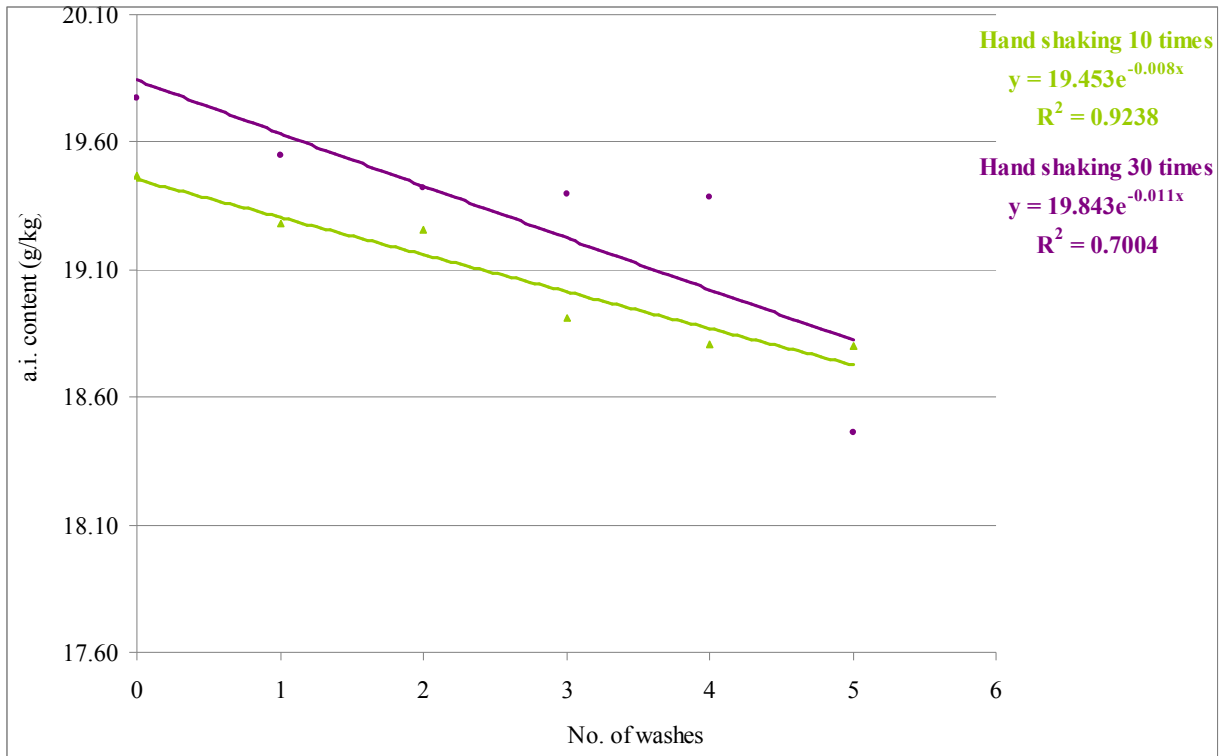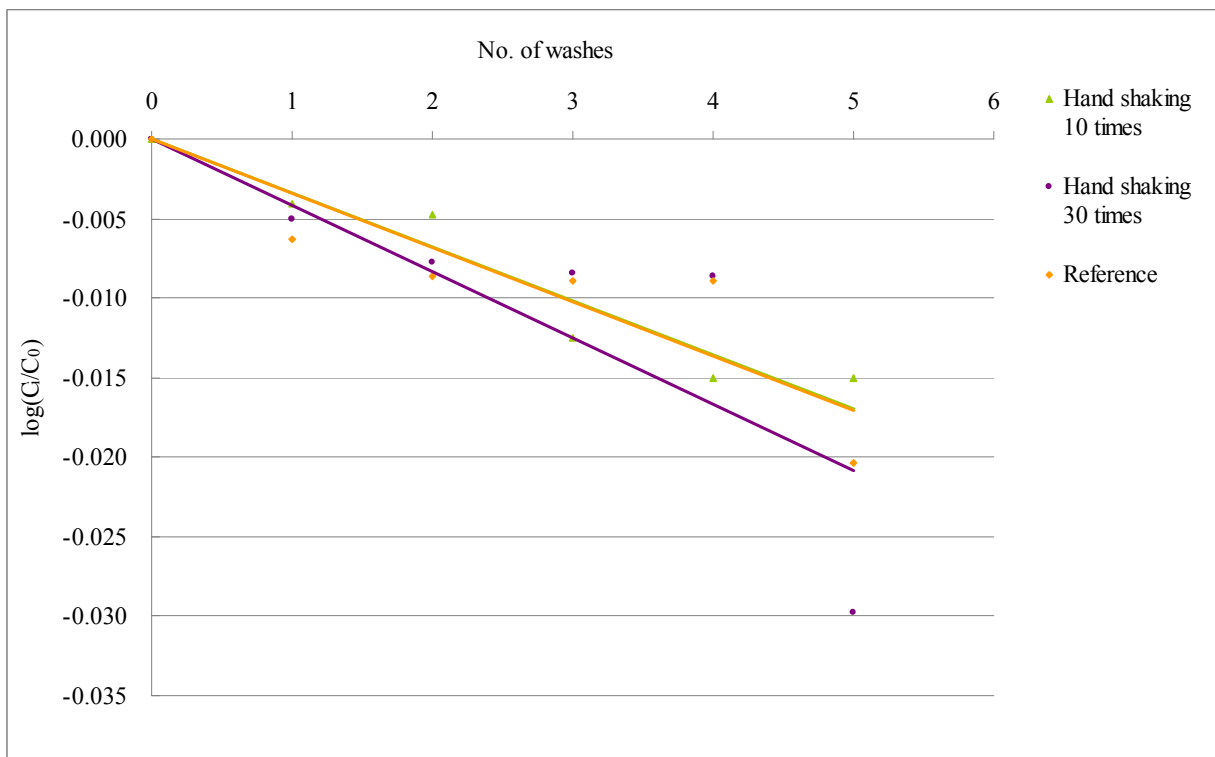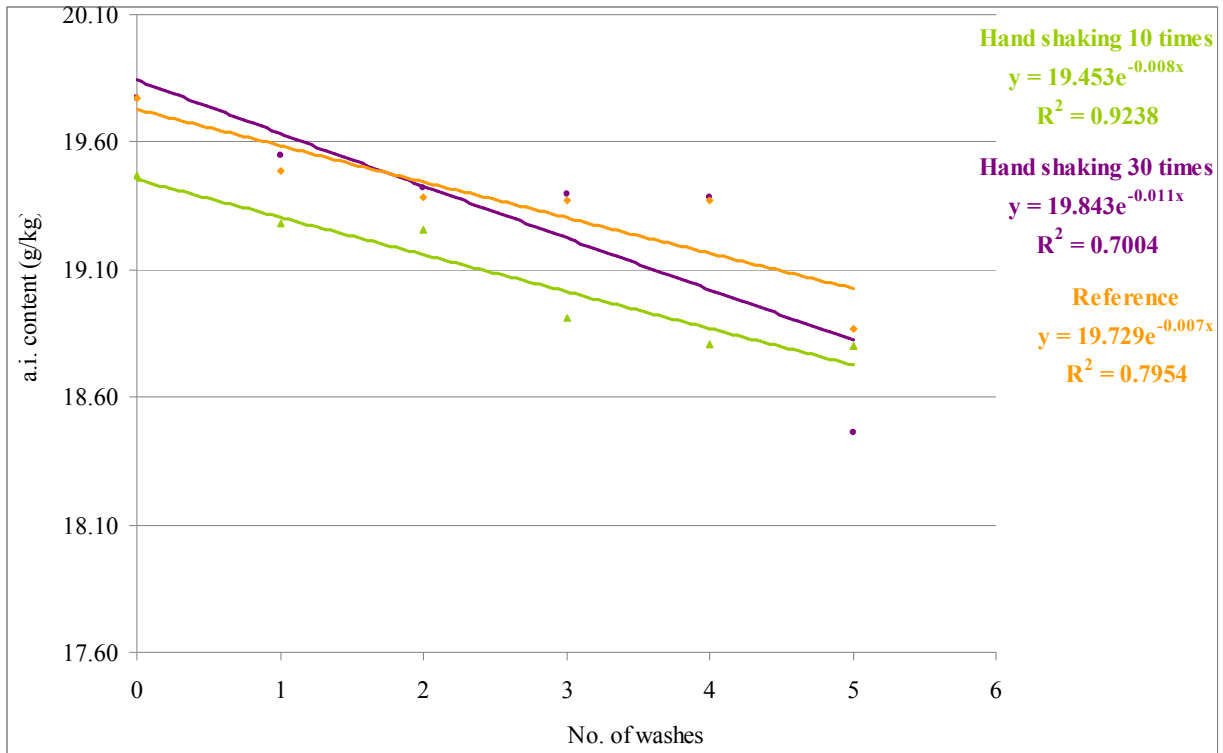
6. **Interpretation of results and conclusions**

The active ingredient content measured on 3 individual unwashed pieces of Olyset® confirms the compliance of the sample with the target dose of 20 g/kg ± 3 g/kg, the good precision of the analytical CIPAC methods and also the acceptable within net homogeneity (RSD = 0.7 and 1.7).

The active ingredient content measured on the 3 individual pieces for each washing cycle is homogeneous indicating an acceptable homogeneity of the washing method (RSD ranging from 0.5 to 2.1) and this RSD is not correlated with the number of washing. The proposed CIPAC washing method (washing / rinsing movement) has therefore an acceptable repeatability.

Concerning the horizontal roller, there is a correlation between the speed of rotation and the power of washing. The high rotating speed is required to gain the same result to the referential result. (See Appendix 1.)

Concerning the hand shaking, there is a correlation between the number of the hand shaking and the power of washing. The result of the 10 times of inversion is closer than that of the 30 times of inversion as compared to the result of the referential result.

However, the differences in average active ingredient content between the 4 objects tested (horizontal roller low speed and high speed, hand shaking 10 times and 30 times) is very small, indicating that the wash resistance index is not significantly affected by the difference of the washing movements.

Appendix 1

LN Detergent Movement	Wash	Active ingredient content (g/kg) Mean	Wash resistance index (% of wash 0)	Log(C_i/C_0)	
Olyset®	0	19.77		0.000	Slope (=log(wr))
	1	19.49	98.6	-0.006	-0.0031
Savon de Marseille	2	19.38	98.0	-0.009	Correl
2 g/L	3	19.37	98.0	-0.009	-0.892
Reciprocal	4	19.37	98.0	-0.009	
Shaker	5	18.87	95.4	-0.020	
Olyset®	0	19.47		0.000	Slope (=log(wr))
	1	19.30	99.1	-0.004	-0.0027
CIPAC washing agent	2	19.13	98.3	-0.008	Correl
8 g/L	3	19.13	98.3	-0.008	-0.972
Horizontal rolling	4	18.89	97.0	-0.013	
60 rpm	5	18.88	97.0	-0.013	
Olyset®	0	19.47		0.000	Slope (=log(wr))
	1	19.23	98.8	-0.005	-0.0024
CIPAC washing agent	2	18.94	97.3	-0.012	Correl
8 g/L	3	18.94	97.3	-0.012	-0.864
Horizontal rolling	4	18.92	97.2	-0.012	
210 rpm	5	18.91	97.1	-0.013	
Olyset®	0	19.47		0.000	Slope (=log(wr))
	1	19.28	99.1	-0.004	-0.0033
CIPAC washing agent	2	19.26	98.9	-0.005	Correl
8 g/L	3	18.91	97.2	-0.013	-0.961
Hand shaking	4	18.81	96.6	-0.015	
10 times	5	18.80	96.6	-0.015	
Olyset®	0	19.77		0.000	Slope (=log(wr))
	1	19.55	98.9	-0.005	-0.0046
CIPAC washing agent	2	19.42	98.2	-0.008	Correl
8 g/L	3	19.39	98.1	-0.008	-0.837
Hand shaking	4	19.38	98.0	-0.009	
30 times	5	18.46	93.4	-0.030	

Pre-testing of the repeatability of the hand shaking movement for washing/rinsing

1. **Sample**
   - Olyset® : permethrin 20 g/kg long-lasting (incorporated into polyethylene) insecticidal mosquito net (LN).

2. **CIPAC washing agent**
   12 g sodium oleate
   + 8 g polyoxyethylene glycol (25) monostearate
   + 80 mL deionised water

3. **Laboratory**
   - Sumitomo, Japan (Yumiko Kozuki and Tsunehisa Fujita) for the testing of Olyset®.

4. **Study procedure**

4.1. **Sampling for wash resistance index**
   From each side face net, 36 times 3 pieces of 25 cm × 25 cm were cut with scissors in the length of the net, parallel to the lower border and put into a 1 L screw capped glass bottle for determination of wash resistance index after 0, 1, 2, 3, 4 and 5 washes with CIPAC washing agent at 8 g/L (3 pieces for each wash cycle) (= 36 samples of 3 pieces for each net).

4.2. **Calculation of wash resistance index**
   Modified WHO washing procedure (= future CIPAC washing method)
   [3 determinations per sample]
   - Washing [3] of 1 individual piece of 25 cm × 25 cm with 500 mL detergent solution.
   - Rinsing 2 times with 500 mL deionized water in a similar way to washing process.
   - Drying on a line at ambient temperature protected from direct sunlight for 30 minutes and then at 40 °C ± 2 °C in the dark for a time period of 22 ± 2 hours before the next washing.
   - After the wash cycles, storage of the piece into an aluminium foil in a freezer at 4 °C (± 3 °C).

   [3] The following movements were compared:
   - Hand shaking at 30 °C ± 2 °C by inverting the 1 litre bottle 10 times
   - Hand shaking at 30 °C ± 2 °C by inverting the 1 litre bottle 30 times

   Determination of active ingredient content
   After the washing procedure, determination of active ingredient content in each individual piece [1 determination for each individual piece]. Results were expressed as g active ingredient per kg netting material. The mean and RSD of the 3 pieces for each wash cycle were calculated and the wash resistance index was calculated for each wash cycle using the equation for a free migration stage behaviour (see table below).
   Methods for active ingredient content

- Olyset® : CIPAC method 331/LN/M/3 (permethrin in incorporated nets), CIPAC Handbook M, page 159.

5. Results of analysis for Olyset®

LN Movement	Wash	Active ingredient content (g/kg)					Wash resistance index (% of wash 0)	Average wash resistance index (% at each wash) (**)
		A (*)	B (*)	C (*)	Mean	RSD (%)		
Olyset®	0	19.7	19.1	19.6	**19.47**	1.7		
	1	19.2	19.3	19.4	**19.28**	0.4	**99.1**	**99.1**
Hand shaking	2	19.3	19.1	19.4	**19.26**	1.0	**98.9**	**99.5**
10 times	3	18.7	19.1	18.9	**18.91**	0.9	**97.2**	**99.0**
	4	19.0	19.0	18.4	**18.81**	2.0	**96.6**	**99.1**
First try	5	18.7	18.9	18.8	**18.80**	0.6	**96.6**	**99.3**
Olyset®	0	19.8	20.3	19.7	**19.91**	1.6		
	1	20.0	19.7	20.1	**19.90**	1.1	**99.9**	**99.9**
Hand shaking	2	19.5	19.6	19.5	**19.51**	0.3	**98.0**	**99.0**
10 times	3	19.0	19.4	19.0	**19.16**	1.2	**96.2**	**98.7**
	4	18.8	19.0	19.6	**19.14**	2.3	**96.1**	**99.0**
Second try	5	19.3	19.0	19.0	**19.12**	0.8	**96.0**	**99.2**
Olyset®	0	19.8	20.3	19.7	**19.91**	1.6		
	1	20.0	19.2	19.7	**19.63**	1.9	**98.6**	**98.6**
Hand shaking	2	19.7	19.5	19.1	**19.41**	1.5	**97.5**	**98.7**
10 times	3	19.3	19.5	19.4	**19.41**	0.6	**97.5**	**99.2**
	4	19.5	19.1	19.5	**19.36**	1.1	**97.2**	**99.3**
Third try	5	19.0	18.9	19.3	**19.07**	1.0	**95.7**	**99.1**

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

(**)**Free migration stage behaviour**

Average wash resistance index = $\sqrt[n]{(t_n/t_0)}$

where t_n = active ingredient total content after wash n, g/kg

t_0 = active ingredient total content at wash 0 (pre-washing), g/kg

n = number of washes.

**Hand shaking 10 times
First try**
$y = 19.453e^{-0.008x}$
$R^2 = 0.9238$

**Hand shaking 10 times
Second try**
$y = 19.932e^{-0.010x}$
$R^2 = 0.8764$

**Hand shaking 10 times
Third try**
$y = 19.828e^{-0.007x}$
$R^2 = 0.9035$

Reference
$y = 19.729e^{-0.007x}$
$R^2 = 0.7954$

LN Movement	Wash	Active ingredient content (g/kg)					Wash resistance index (% of wash 0)	Average wash resistance index (% at each wash) (**)
		A (*)	B (*)	C (*)	Mean	RSD (%)		
Olyset®	0	20.3	20.3	20.0	**20.16**	0.9		
	1	19.7	19.5	20.0	**19.74**	1.2	**97.9**	**97.9**
Hand shaking	2	18.9	19.5	19.3	**19.25**	1.7	**95.5**	**97.7**
30 times	3	19.2	19.5	19.0	**19.24**	1.2	**95.4**	**98.4**
First try	4	18.9	19.1	19.3	**19.11**	1.0	**94.8**	**98.7**
Olyset®	0	20.3	20.3	20.0	**20.16**	0.9		
	1	19.4	19.4	19.0	**19.25**	1.2	**95.4**	**95.4**
Hand shaking	2	19.4	19.4	19.8	**19.53**	1.3	**96.9**	**98.4**
30 times	3	19.4	19.6	19.5	**19.48**	0.4	**96.6**	**98.9**
Second try	4	19.2	19.1	19.3	**19.19**	0.5	**95.2**	**98.8**
Olyset®	0	20.3	20.3	20.0	**20.16**	0.9		
	1	19.6	19.8	19.8	**19.71**	0.7	**97.7**	**97.7**
Hand shaking	2	19.4	19.8	19.6	**19.60**	1.2	**97.2**	**98.6**
30 times	3	19.5	19.5	19.6	**19.51**	0.3	**96.7**	**98.9**
Third try	4	19.3	19.4	19.2	**19.27**	0.6	**95.6**	**98.9**

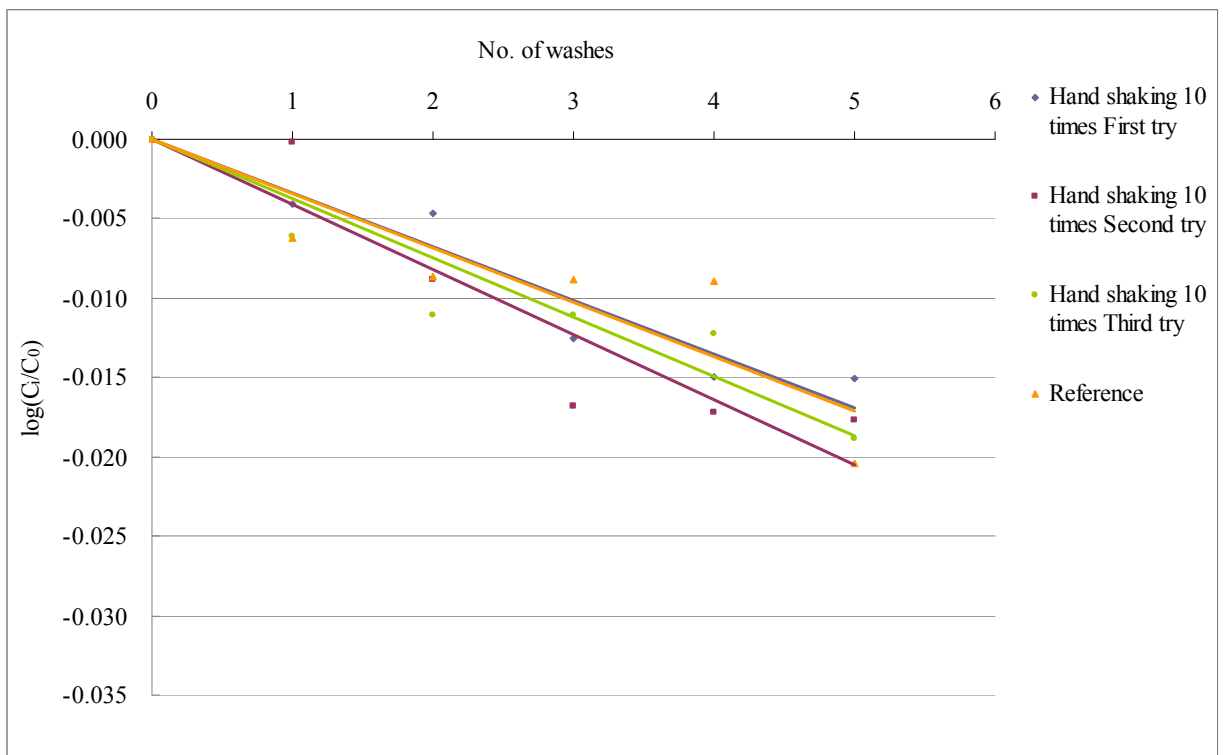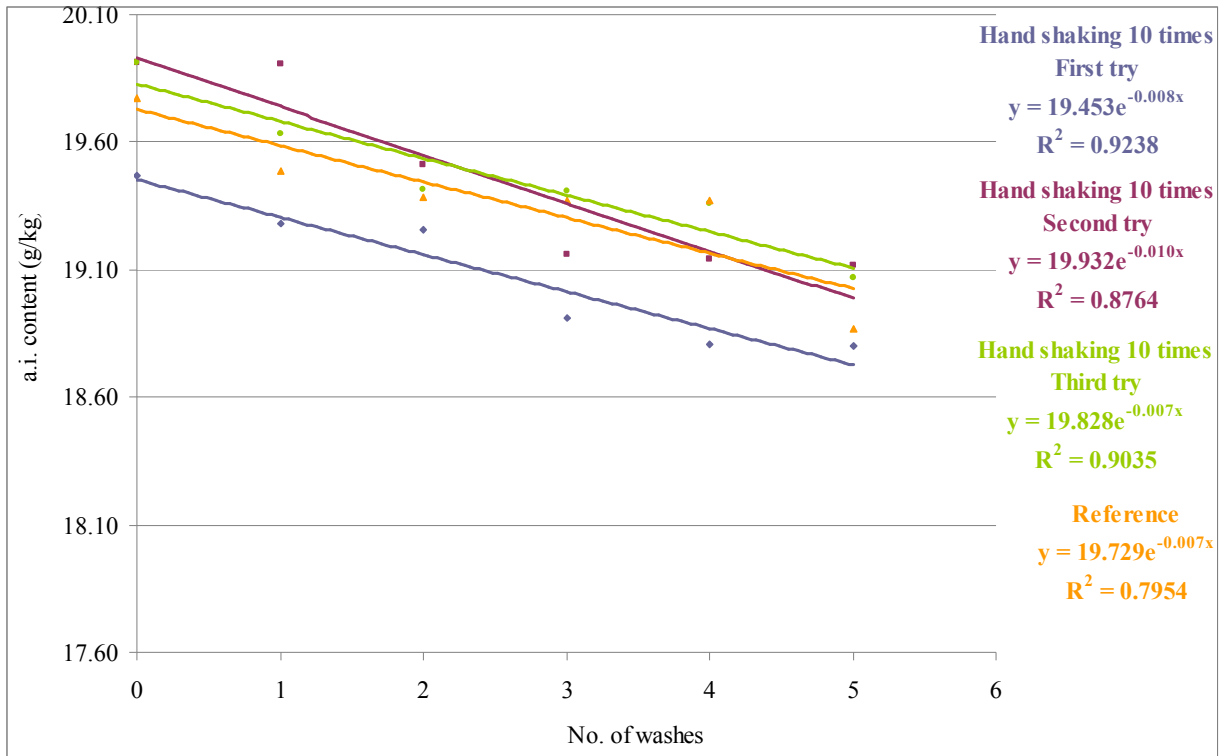(*) Each result is the mean of 2 chromatographic injections (duplicate injections).

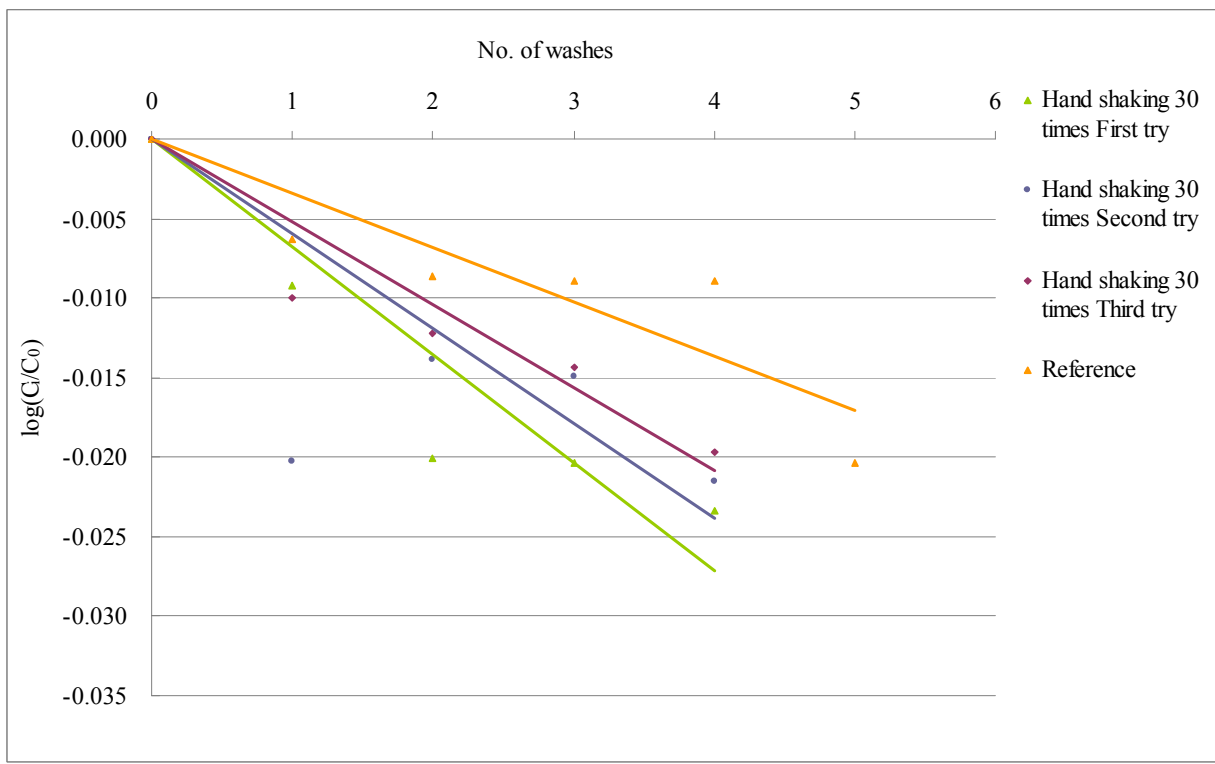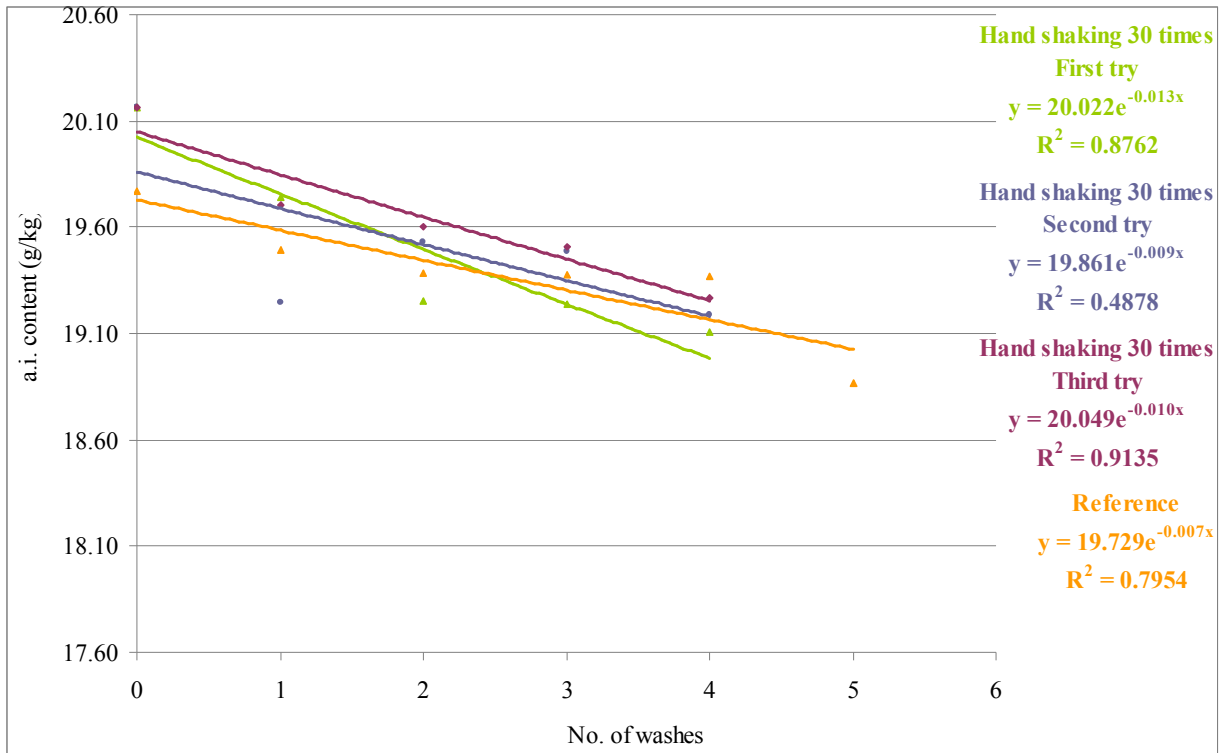(**)**Free migration stage behaviour**

Average wash resistance index = $\sqrt[n]{(t_n/t_0)}$

where t_n = active ingredient total content after wash n, g/kg

t_0 = active ingredient total content at wash 0 (pre-washing), g/kg

n = number of washes.

Graph 1 (top): y-axis "a.i. content (g/kg)", x-axis "No. of washes"

**Hand shaking 30 times
First try**
$y = 20.022e^{-0.013x}$
$R^2 = 0.8762$

**Hand shaking 30 times
Second try**
$y = 19.861e^{-0.009x}$
$R^2 = 0.4878$

**Hand shaking 30 times
Third try**
$y = 20.049e^{-0.010x}$
$R^2 = 0.9135$

Reference
$y = 19.729e^{-0.007x}$
$R^2 = 0.7954$

Graph 2 (bottom): y-axis "$\log(C_i/C_0)$", x-axis "No. of washes"

Legend:
▲ Hand shaking 30 times First try
● Hand shaking 30 times Second try
◆ Hand shaking 30 times Third try
▲ Reference

6. <u>**Interpretation of results and conclusions**</u>

The active ingredient content measured on 3 individual unwashed pieces of Olyset® confirms the compliance of the sample with the target dose of 20 g/kg ± 3 g/kg, the good precision of the analytical CIPAC methods and also the acceptable within net homogeneity (RSD = 0.9, 1.6 and 1.7).

The active ingredient content measured on the 3 individual pieces for each washing cycle is homogeneous indicating an acceptable homogeneity of the washing method (RSD ranging from 0.3 to 2.3) and this RSD is not correlated with the number of washing. The proposed CIPAC washing method (washing / rinsing movement) has therefore an acceptable repeatability.

When it is compared to the referential result, Olyset® is washed / rinsed by hand shaking method inverting 10 times, the result gained is similar in respect of the ability of washing. (See Appendix 2.) Concerning Olyset® is washed / rinsed by hand shaking method inverting 30 times, the difference is slightly found.

However, the difference in average active ingredient content between the 6 objects tested is very small, indicating that the wash resistance index is not significantly affected by the difference of the inverting times of the hand shaking movements.

Appendix 2

LN Movement	Wash	Active ingredient content (g/kg) Mean	Wash resistance index (% of wash 0)	Log(C_i/C_0)	
Olyset®	0	19.47		0.000	Slope (=log(wr))
	1	19.28	99.1	-0.004	-0.0033
Hand shaking	2	19.26	98.9	-0.005	Correl
10 times	3	18.91	97.2	-0.013	-0.961
	4	18.81	96.6	-0.015	
First try	5	18.80	96.6	-0.015	
Olyset®	0	19.91		0.000	Slope (=log(wr))
	1	19.90	99.9	-0.000	-0.0042
Hand shaking	2	19.51	98.0	-0.009	Correl
10 times	3	19.16	96.2	-0.017	-0.936
	4	19.14	96.1	-0.017	
Second try	5	19.12	96.0	-0.018	
Olyset®	0	19.91		0.000	Slope (=log(wr))
	1	19.63	98.6	-0.006	-0.0032
Hand shaking	2	19.41	97.5	-0.011	Correl
10 times	3	19.41	97.5	-0.011	-0.951
	4	19.36	97.2	-0.012	
Third try	5	19.07	95.7	-0.019	
Olyset®	0	20.16		0.000	Slope (=log(wr))
	1	19.74	97.9	-0.009	-0.0058
Hand shaking	2	19.25	95.5	-0.020	Correl
30 times	3	19.24	95.4	-0.020	-0.936
First try	4	19.11	94.8	-0.023	
Olyset®	0	20.16		0.000	Slope (=log(wr))
	1	19.25	95.4	-0.020	-0.0038
Hand shaking	2	19.53	96.9	-0.014	Correl
30 times	3	19.48	96.6	-0.015	-0.698
Second try	4	19.19	95.2	-0.022	
Olyset®	0	20.16		0.000	Slope (=log(wr))
	1	19.71	97.7	-0.010	-0.0044
Hand shaking	2	19.60	97.2	-0.012	Correl
30 times	3	19.51	96.7	-0.014	-0.956
Third try	4	19.27	95.6	-0.020	