

# THE INFLUENCE OF WASHING PRACTICES ON THE EFFICACY OF DELTAMETHRIN LONG LASTING INSECTICIDE TREATED MOSQUITO NETS (ITNs) USING GLASS TUNNEL TEST METHOD

Damar Tri Boewono<sup>1</sup>, Widiarti<sup>1</sup>, Mujiono<sup>1</sup>

**Abstrak.** Suatu penelitian untuk mengetahui aktivitas residu dan respon iritabilitas Perma-Net® long lasting-insecticide-treated mosquito nets (ITNs) deltamethrin 55 mg/m<sup>2</sup> setelah pencucian, dilakukan di BPVRP Salatiga, bulan Oktober-November 2004 dengan metoda uji *glass tunnel*. Kelambu PermaNet® dicuci dengan sabun padat (0, 6, 9, 12, 18, 21 dan 24 kali) sebagai perlakuan. Pencucian kelambu dilakukan tiga kali (secara individual) di dalam *blue cap bottle* (volume 1 liter), diisi air 500 ml dan digoyang pada *shaker* selama 20 menit, dibilas 2 kali dan dikeringkan di tempat terlindung cahaya matahari, pada posisi datar. Evaluasi dilakukan terhadap kematian nyamuk uji (*Anopheles maculatus*) serta respon iritabilitas (jumlah nyamuk uji dapat melewati lubang pada kelambu berinsektisida) dan jumlah nyamuk uji kenyang darah marmot yang dipasang sebagai umpan. Hasil uji menunjukkan bahwa penurunan aktivitas residu insektisida deltamethrin sejalan dengan frekuensi pencucian ITNs. Rata-rata kandungan deltamethrin setelah dicuci 24 kali adalah 8,78 mg/m<sup>2</sup> (berkurang 84,24% atau 1,93 mg/m<sup>2</sup> setiap pencucian) dan kematian nyamuk uji 80,0%, dari jumlah 100 ekor. Uji *glass tunnel* menunjukkan bahwa, kematian *An. maculatus* pada kelambu (0 dan 6 kali pencucian) tidak berbeda secara bermakna, akan tetapi berbeda nyata setelah 12, 18, 21 dan 24 kali pencucian. Kematian nyamuk uji pada kelambu yang telah dicuci 21 dan 24 kali, menurun menjadi 67,97% dan 55,15% (tidak efektif menurut standar WHO 70%) dan tidak berbeda nyata dengan kelambu dicuci 18 kali. Respon iritabilitas tidak berbeda nyata berdasarkan jumlah pencucian. Efek repelen PermaNet® tidak cukup besar, nampak dari jumlah nyamuk melewati lubang pada kelambu uji 14,17-30,91% (±1,63%) ditemukan kenyang darah.

*Key words* : *glass tunnel test*; *ITNs deltamethrin*; *washing frequency*; *An. maculatus*.

## INTRODUCTION

Bed nets have long been used to protect people from mosquitoes and other biting insects. The traditional bed net loses its effectiveness when it becomes torn because insects can enter through holes. One way to overcome this disadvantage is to treat bed nets with an insecticide. Deltamethrin and permethrin are pyrethroids recommended for impregnation of bed nets by the WHO Expert Committee on Vector Biology and Control<sup>(1)</sup>.

Pyrethroid treated bed nets have been shown to cause a decline on malaria trans-

mission<sup>(2, 3, 4)</sup>. These investigations were based on nets that were dipped in a water emulsion of a pyrethroid. Such nets lose their efficacy by washing practices or after about 6-12 months of use, depending on the pyrethroid and product<sup>(2, 5, 6, 7)</sup>. Net dipping and especially reimpregnations is proven to be costly and ineffective unless it is intensively encouraged<sup>(8, 9, 10)</sup> and use no charge as in Vietnam. When net dipping is changed from being a free service to self-financed, retreatment rates fell near to zero in Gambia<sup>(11)</sup> and Kenya<sup>(12)</sup>. To overcome these problems, the WHO guided Roll Back Malaria initiative to use net distribu-

<sup>1</sup> Vector and Reservoir Control Research Unit, Salatiga

tion. Indeed, this condition has encouraged the industry to develop pre-treated nets, which consider unnecessary for retreated process. Therefore the nets can continually be used.

PermaNet® (Vestergaard-Frandsen) and Olyset. (Sumitomo Chemicals) products, were the first nets of polyethylene and polyester developed to meet these targets. A prototype of PermaNet® made in early 2000 was tested in Burkina Faso and did not show sufficient wash resistance<sup>(13)</sup>. A few months later, an improved version was put into production, five of these bed nets were tested in Colombia. The nets were washed repeatedly and then evaluated in the laboratory in comparison with locally dipped nets. The residual activity of the nets after the washing process was estimated in bioassay that showed the dipped nets failed to give 80% mortality with 30 minutes exposure after 1-2 washings, whereas PermaNet® resisted 20 washings whether there were carried out as 3 washings per week or one per week over 20 weeks<sup>(7)</sup>.

The objective of the study is to evaluate the residual activity and repellence effect of PermaNet® (long lasting-insecticide-treated mosquito nets/ITNs) of Deltamethrin 55 mg/m<sup>2</sup> after washing practices, measured in terms of mortality, respond of irritability (blood feeding and the reduction of penetration malaria vector through the holes of the net).

## II. MATERIALS AND METHODS

The study was conducted in the Vector and Reservoir Control Research Unit, (VRCRU), Salatiga Central Java Province, Indonesia in October-November 2004, using malaria vector *An. maculatus* in three replicates.

### 1. Materials:

Susceptibility test kit (WHO standard), sucking tube, plastic cups, cotton wool, rubber bands, glucose solution, guinea-pigs, glass tunnel, detergent, alcohol, PermaNet®: 20x20 cm<sup>2</sup> (polyester multi-filament 100 denier, impregnated with deltamethrin 55 mg/m<sup>2</sup>, mesh 156 from Vestergaard Disease Control Textile), special treatments: (0, 6, 9, 12, 18, 21 and 24 washings); female mosquitoes *An. maculatus* 3-4 days old and glucose fed (colonies maintained in the insectaries of the VRCRU)

## 2. Methods

### a. Net washing practices

The nets washing were conducted individually using blue cap bottle (1000 ml volume). The bottles were filled with 500 ml of clean water (1 gram of solid soap was added in each bottle) and shaken for 20 minutes. Washed net was rinsed three times using same method and gently squeezed with clean water into the blue cap bottle and shaken for 10 minutes each time. After being washed, the nets were laid flat in the shade and allowed to dry.

After 24 washings, four net samples were sent to the Disease Control Textile, Hanoi, Vietnam, for chemical analysis to evaluate the residuals concentration of deltamethrin on the PermaNet®.

### b. Susceptibility test

Susceptibility test of female mosquitoes against insecticides was conducted using WHO test kit. Impregnated paper of deltamethrin 0.05% and DDT 4% were used for the evaluation with three replicates for each tested insecticide.

A number of 20 mosquitoes (*An. maculatus*) sugar fed, 3-4 days old, were placed in clean cups the day before the test. Tested mosquitoes were transferred to the holding (green marked) WHO susceptibility test tube. Inner surfaces of the exposure (red marked) tubes were lined with impregnated paper of deltamethrin 0.05 % or DDT 4%. Tested mosquitoes were then blown to the exposed tube (for 60 minutes exposure). After exposure they were gently blown out into the holding tube. The number of knockdown was recorded after 60 minutes exposure and the mortality reading was noted 24 hours later (WIO, 2005).

### c. Tunnel test

The basic equipment consists of a tunnel (square 25 x 25 cm) made of glass, 75 cm length (see Annex 1, Figure 3). At one third of the length, a disposable cardboard frame is placed with the treated mosquito net sample. The surface of mosquito net samples "available" were 400 cm<sup>2</sup> (20 x 20 cm<sup>2</sup>) with 9 holes of 1 cm in diameter. Within the small side, a guinea pig was placed (unable to move but available for biting). At the end of each side of the tunnel, a 30 cm square cage is fitted, covered with polyester netting. In the cage placed at the end of the long side, 100 females *An. maculatus* were introduced at 6 PM. Females were free to fly inside the cage and move towards the bait. In order to reach that bait, they have to flow along the treated net, locate the holes and pass it through. Thus, they will be able to bite the guinea-pig easily.

After the blood meals, females usually fly to the cage at the end of this compartment and rest. The next morning at 9 AM, females tested mosquitoes were removed separately from each side of glass cage, maintained for 24 hours for mortality observation. Females' exposure mosquitoes were maintained in the laboratory and in a climatic condition at 28° and 80% RH in plastic cups, provided with glucose solution (no more than 25 females per cup).

For each test series, 5 cages were used in parallel, one as a control (UTC=Untreated Control) and 4 as treated. The tests were conducted in triplicates, each time with different rearing batches. After every test series (1 UTC and 4 treated were allocated randomly), glass tunnels were cleaned with both detergent and alcohol. The tested mosquito net samples were removed from the cages and washed with detergent.

Tested mosquitoes in the tunnel test were measured for following effects:

1. Mortality: was measured both as immediate mortality (observed at 9 AM in the morning) and delayed mortality (observed 24 hours after) as well as over all mortality (immediate + delayed)
2. Respond of irritability
  - ❖ Repellence: expressed by the number (%) of females which were able to fly through the treated net holes (repellence dramatically reduce this number)
  - ❖ Blood feeding: was measured by counting females (%)

which were viewable to take a blood from the guinea-pig.

The data was analyzed using computer programmes, based on the frequency of nets washing accordingly: Analysis Varian of the mean mortality difference and the respond of irritability, using SPSS programme, version 11.0.

### III. RESULTS

Susceptibility of *An. maculatus* against deltamethrin 0.05% and DDT 4.0%

showed 100.0% mortality (Table 1). Whereas, bioassay tests of deltamethrin mosquito nets (after 21 and 24 washings) showed 80.0-83.0% mortality (Table 2).

Tested mosquitoes mortalities according to the number of net washings are presented in Table 3 and Figure 1. It can be observed that the *An. maculatus* mortality for 18 washings and less were yet above 80.0%. However, for 21 and 24 washings were already less than 70.0%. The original (0 washing) concentration of deltamethrin on the PermaNet<sup>®</sup> was 55 mg/m<sup>2</sup>, whereas the mean of 24 washings was  $8.78 \pm 1.12$  (SD) mg/m<sup>2</sup> (Table 4).

**Table 1. Susceptibility of *An. maculatus* against deltamethrin 0.05% and DDT 4.0% <sup>(1)</sup>**

Insecticide	KD 50 <sup>(2)</sup>	KD after 60'' contact (%)	Effective mortality (%)	Functional mortality (%)
Deltamethrin 0.05%	15 min	100.0	100.0	100.0
DDT 4%	25 min	100.0	100.0	100.0

(2) KD = Knock down

(1) The test was conducted in three replicates (20 tested mosquitoes each)

**Table 2. Bioassay test of *An. maculatus* on long lasting insecticide treated nets, sample 114 (21 washings) & 38 (24 washings), conducted in (3 replicates)<sup>#</sup>**

Net Sample tested	KD after 60'' contact (%)	Effective mortality (%)	Functional mortality (%)
Control	0.0	4.0	4.0
Samples 114 (21 washings)	95.0	68.0	80.0
Samples 38 (24 washings)	100.0	83.0	83.0

# Test conducted using WHO test kit 15 mosquitoes each cone (exposure time for 3 minutes) (see Annex 1)

**Table 3. Residual deltamethrin of four PermaNet<sup>®</sup> samples after 24 washed <sup>1</sup>**

Net Sample	Residual <sup>1</sup> deltamethrin (mg/m <sup>2</sup> )	Residual <sup>2</sup> deltamethrin (mg/m <sup>2</sup> )
1	12.5	10.3
2	8.8	8.1
3	9.7	7.8
4	7.4	8.9
Mean	$9.6 \pm 2.1$ (SD)	$8.78 \pm 1.12$ (SD)

1). Determined by VDCT, Hanoi, Vietnam; Ole Skovmand Study, 2004; 2). Present Study

**Table 4. Tunnel test evaluation of the mortality and respond irritability of PermaNet® according to the washing frequencies**

		REPLICATE 1			REPLICATE 2			REPLICATE 3			Average (3 replicates)
		Control	Treated	Corrected by control	Control	Treated	Corrected by control	Control	Treated	Corrected by control	
Deltamethrin 25 mg/m2	Number female tested	96	102								
	Mortality (%)	5	94.12	94.12							
	Pass through the net (%)	63	23.53	23.53							
	Blood fed (%)	15	0.00	0.00							
Ref. 107: 0 washing	Number female tested	96	103		98	101		104	100		
	Mortality (%)	6	90.29	92.55	6	92.08	92.55	7	96.00	95.70	93.60
	Pass through the net (%)	63	31.07	31.07	36	32.67	32.67	27	29.00	29.00	30.91
	Blood fed (%)	15	0.00	0.00	42	0.00	0.00	21	0.00	0.00	0.00
Ref. 37: 6 washings	Number female tested	96	94		97	105		104	102		
	Mortality (%)	6	96.81	90.43	6	90.48	94.68	7	92.16	93.55	92.88
	Pass through the net (%)	63	9.57	9.57	53	18.10	18.10	27	26.47	26.47	18.05
	Blood fed (%)	15	0.00	0.00	21	0.00	0.00	21	0.00	0.00	0.00
Ref. 27: 12 washings	Number female tested	96	93		97	99		104	97		
	Mortality (%)	6	89.25	80.85	6	91.92	90.43	7	88.66	84.95	85.41
	Pass through the net (%)	63	29.03	29.03	53	14.14	14.14	27	28.87	28.87	24.01
	Blood fed (%)	15	0.00	0.00	21	1.01	1.01	21	0.00	0.00	0.34
Ref. 84: 18 washings	Number female tested	96	102		97	104		104	95		
	Mortality (%)	6	84.31	84.04	6	83.65	86.17	7	87.37	81.72	83.98
	Pass through the net (%)	63	13.73	13.73	53	18.27	18.27	27	10.53	10.53	14.17
	Blood fed (%)	15	0.00	0.00	21	0.96	0.96	21	2.11	2.11	1.02
Ref. 44: 21 washings	Number female tested	101	101		102	98		99	107		
	Mortality (%)	7	70.30	66.67	7	73.47	69.89	5	64.49	67.37	67.98
	Pass through the net (%)	69	9.90	9.90	61	23.47	23.47	54	28.97	28.97	20.78
	Blood fed (%)	36	0.99	0.99	29	2.04	2.04	19	1.87	1.87	1.63
Ref. 11: 24 washings	Number female tested	101	96		102	97		99	102		
	Mortality (%)	7	58.33	51.61	7	62.89	58.06	5	56.86	55.79	55.16
	Pass through the net (%)	69	5.21	5.21	61	28.87	28.87	54	18.63	18.63	17.57
	Blood fed (%)	36	0.00	0.00	29	1.03	1.03	19	1.96	1.96	1.00

#### IV. DISCUSSION

The susceptibility test study showed that *An. maculatus* (3 years maintained in the VRCRU laboratory) is still susceptible against DDT (4.0%) and deltamethrin 0.05% (Table 1). The reduction of deltamethrin residuals in PermaNet<sup>®</sup> (ITNs) when tested by means of washing experiments in the laboratory of VRCRU Salatiga was very similar to the decline found in the 4 samples of the Colombian study<sup>(7)</sup>. In the laboratory an experiment with one week interval between the processes of net washing, reveals the declining of deltamethrin concentration at 7.33% per wash.

Previous study conducted in the laboratory of VRCRU Salatiga, revealed that after 23 net washings, the residual concentration of its initial level was declining from 55 mg deltamethrin/m<sup>2</sup> to 9.6 mg/m<sup>2</sup><sup>(15)</sup>. Present study found that the concentration of deltamethrin was less compared to the previous study. In addition, a level of 8.78 mg deltamethrin/m<sup>2</sup> was identified after 24 washings (Table 3). Whereas, the average residual lost of deltamethrin concentration was 3.5% (1.93 mg/m<sup>2</sup>) at each wash.

Study of bioassay tests of deltamethrin long lasting insecticide treated nets (after 21 and 24 washings), caused 80.0% and 83.0% mortality (Table 2). These results indicate that the nets are still effective to kill the mosquitoes (WHO standard 70.0% mortality).

Other study of Olyset polyethylene net (impregnated with permethrin 2% w/w), which had been conducted in the laboratory of Institute for Medical Research, Kuala Lumpur, Malaysia, revealed that percentage mortality of *An. maculatus* exposed to Olyset nets after washed four times with soap and water was 86.7%<sup>(16)</sup>.

The villagers in Columbia reported that PermaNet<sup>®</sup> can control *Anopheles* mosquitoes for at least 3 years as measured in bioassays, more than 80.0% mortality (Annex 1, Figure 2). Using the susceptible laboratory colony strain *An. maculatus* and shorter exposure time of 3 minutes (WHO standard 30 minutes), the lower limit of deltamethrin for at least 80.0% functional mortality is 3-5 mg/m<sup>2</sup><sup>(14)</sup>. Other result reported that 43.5-48.3% anophelines mortality at 3 mg deltamethrin/m<sup>2</sup> was found in hut studies<sup>(5)</sup>.

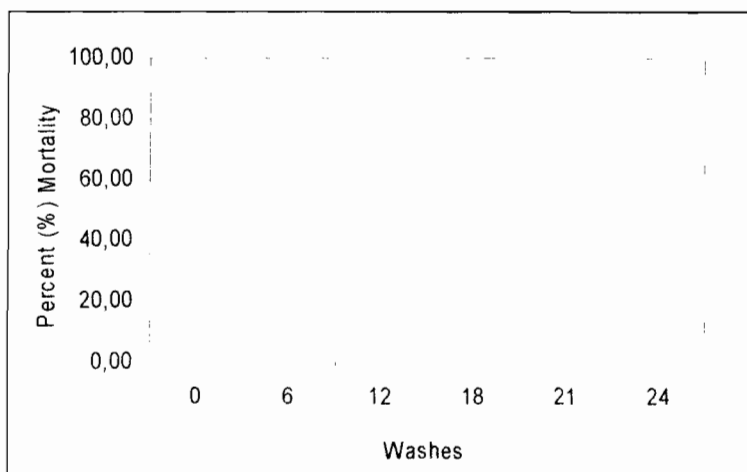


Figure 1. Mortality of tested mosquitoes (*An. maculatus*) in the tunnel test according to the number of net washing

Using the above-mentioned calculation of loss on the deltamethrin due to the washing process, the nets should be effective for 4 (using WHO standards 80% mortality) washes against *Anopheles*. The original study in Columbia showed that the efficacy against local *Culex* sp was pronounced: 99.0% after 4 washes that dropped to 71.0% after 20 washes. The less pronounced effect on *Culex* of pyrethroid impregnated nets has also been found in other studies<sup>(5)</sup>.

The tunnel test study using PermaNet<sup>®</sup> found that the average mortalities of tested mosquitoes were 93.60% (0 wash), declined to 92.88% (6 washes); 85.41% (12 washes); 83.97 (18 washes). The mortality of tested mosquitoes (PermaNet<sup>®</sup> 21 and 24 washes) 67.97-55.15% (Table 4, Figure 1), was ineffective (according to WHO standard 70.0%). Mortality of *An. maculatus* for 0 and 6 washes were not significantly different ( $p < 0.05$ ), but they did on 12, 18, 21 and 24 washes ( $p > 0.05$ ). The mortality of tested mosquitoes on PermaNet (which were washed for 21 and 24 times) were not significantly different ( $p < 0.05$ ). However, deltamethrin ITNs in 18 washes were still effective to kill the mosquitoes (83.98%).

### Respond of irritability

The number of mosquitoes which were able to pass through the net holes were not significantly different ( $p < 0.05$ ) according to the washing frequency. Based on these results, repellent effect was low performed by the PermaNet<sup>®</sup>. Related to that, the declining of the mosquitoes that might be able to pass through the net holes was not dramatically reduced. Only few number of blood fed mosquitoes were found (capable to take a blood from the guinea pig) in the second cage. Although,

few number of mosquitoes were able to pass through the PermaNet<sup>®</sup> (after 21 washes) still the result revealed that it was significantly different with those which were done in 0, 6, 12 washes ( $p > 0.05$ ).

### V. ACKNOWLEDGMENTS

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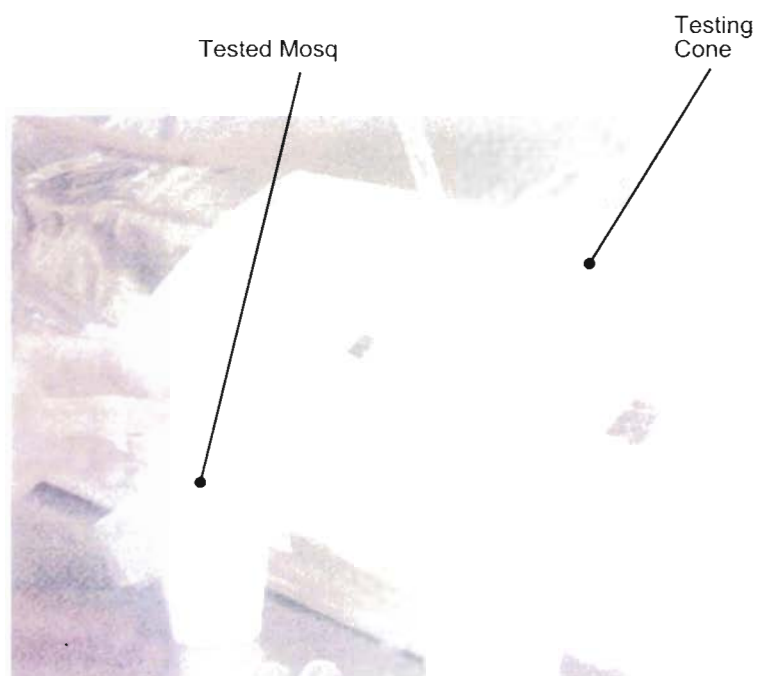
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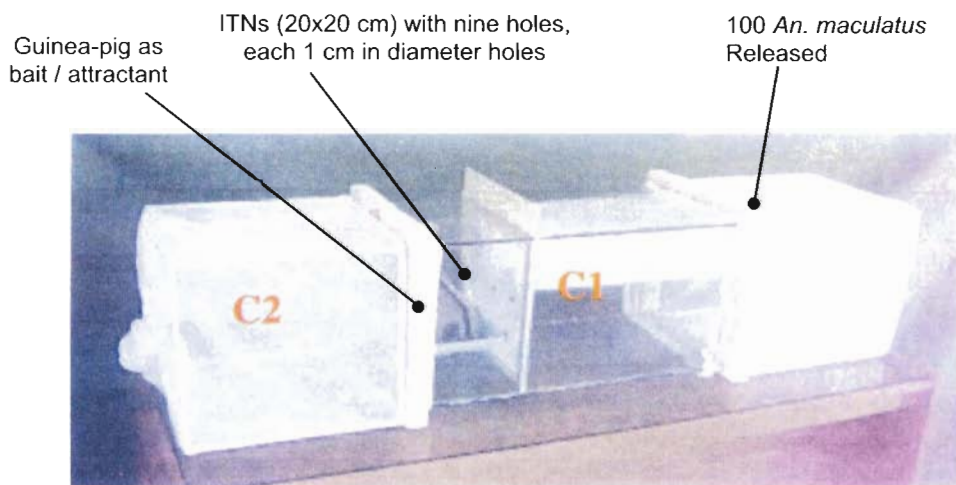


## Annex 1



Taken from: WHO/CDC/WHOPES/GCDPP/2005.11

**Figure 2. Cone bioassay on long lasting impregnated mosquito nets (ITNs)**



**Figure 3. The glass tunnel for the study of the efficacy on insecticide-treated mosquito nets (WHO, 2005)**

**Annex 2.**

**One way**

ANOVA

Mortality Female Tested

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3473.580	5	694.716	83.730	.000
Within Groups	99.565	12	8.297		
Total	3573.145	17			

**Post Hoc Tests**

Multiple Comparisons

Dependent Variable: Female Tested  
LSD

(I) Grade of net's washings	(J) Grade of the net's washings	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 wash	6 washings	.7133	2.3519	.767	-4.4110	5.8377
	12 washings	8.1900*	2.3519	.005	3.0657	13.3143
	18 washings	9.6233*	2.3519	.001	4.4990	14.7477
	21 washings	25.6233*	2.3519	.000	20.4990	30.7477
	24 washings	38.4467*	2.3519	.000	33.3223	43.5710
6 washings	6 washings	-.7133	2.3519	.767	-5.8377	4.4110
	12 washings	7.4767*	2.3519	.008	2.3523	12.6010
	18 washings	8.9100*	2.3519	.003	3.7857	14.0343
	21 washings	24.9100*	2.3519	.000	19.7857	30.0343
	24 washings	37.7333*	2.3519	.000	32.6090	42.8577
12 washings	6 washings	-8.1900*	2.3519	.005	-13.3143	-3.0657
	12 washings	-7.4767*	2.3519	.008	-12.6010	-2.3523
	18 washings	1.4333	2.3519	.554	-3.6910	6.5577
	21 washings	17.4333*	2.3519	.000	12.3090	22.5577
	24 washings	30.2567*	2.3519	.000	25.1323	35.3810
18 washings	6 washings	-9.6233*	2.3519	.001	-14.7477	-4.4990
	12 washings	-8.9100*	2.3519	.003	-14.0343	-3.7857
	18 washings	-1.4333	2.3519	.554	-6.5577	3.6910
	21 washings	16.0000*	2.3519	.000	10.8757	21.1243
	24 washings	28.8233*	2.3519	.000	23.6990	33.9477
21 washings	6 washings	-25.6233*	2.3519	.000	-30.7477	-20.4990
	12 washings	-24.9100*	2.3519	.000	-30.0343	-19.7857
	18 washings	-17.4333*	2.3519	.000	-22.5577	-12.3090
	21 washings	-16.0000*	2.3519	.000	-21.1243	-10.8757
	24 washings	12.8233*	2.3519	.000	7.6990	17.9477
24 washings	6 washings	-38.4467*	2.3519	.000	-43.5710	-33.3223
	12 washings	-37.7333*	2.3519	.000	-42.8577	-32.6090
	18 washings	-30.2567*	2.3519	.000	-35.3810	-25.1323
	21 washings	-28.8233*	2.3519	.000	-33.9477	-23.6990
	24 washings	-12.8233*	2.3519	.000	-17.9477	-7.6990

\* The mean difference is significant at the .05 level.

**Oneway**

## ANOVA

Mortality Female Tested

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	523.218	5	104.644	1.569	.242
Within Groups	800.340	12	66.695		
Total	1323.558	17			

**Post Hoc Tests**

## Multiple Comparisons

Dependent Variable: Female Tested  
LSD

(I) Grade of net's washings	(J) Grade of the net's washings	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 wash	6 washings	12.867	6.6681	.078	-1.6618	27.3952
	12 washings	6.9000	6.6681	.321	-7.6285	21.4285
	18 washings	16.7367*	6.6681	.027	2.2082	31.2652
	21 washings	10.1333	6.6681	.154	-4.3952	24.6618
	24 washings	13.3433	6.6681	.069	-1.1852	27.8718
6 washings	6 washings	-12.8667	6.6681	.078	-27.3952	1.6618
	12 washings	-5.9667	6.6681	.388	-20.4952	8.5618
	18 washings	3.8700	6.6681	.572	-10.6585	18.3985
	21 washings	-2.7333	6.6681	.689	-17.2618	11.7952
	24 washings	.4767	6.6681	.944	-14.0518	15.0052
12 washings	6 washings	-6.9000	6.6681	.321	-21.4285	7.6285
	12 washings	5.9667	6.6681	.388	-8.5618	20.4952
	18 washings	9.8367	6.6681	.166	-4.6918	24.3652
	21 washings	3.2333	6.6681	.636	-11.2952	17.7618
	24 washings	6.4433	6.6681	.353	-8.0852	20.9718
18 washings	6 washings	-16.7367*	6.6681	.027	-31.2652	-2.2082
	12 washings	-3.8700*	6.6681	.572	-18.3985	10.6585
	18 washings	-9.8367	6.6681	.166	-24.3652	4.6918
	21 washings	-6.6033	6.6681	.342	-21.1318	7.9252
	24 washings	-3.3933	6.6681	.620	-17.9218	11.1352
21 washings	6 washings	-10.1333	6.6681	.154	-24.6618	4.3952
	12 washings	2.7333	6.6681	.689	-11.7952	17.2618
	18 washings	-3.2333	6.6681	.636	-17.7618	11.2952
	21 washings	6.6033	6.6681	.342	-7.9252	21.1318
	24 washings	3.2100	6.6681	.639	-11.3185	17.7385
24 washings	6 washings	-13.3433	6.6681	.069	-27.8718	1.1852
	12 washings	-.4767	6.6681	.944	-15.0052	-14.0518
	18 washings	-6.4433	6.6681	.353	-20.9718	8.0852
	21 washings	3.3933	6.6681	.620	-11.1352	17.9218
	24 washings	-3.2100	6.6681	.639	-17.7385	11.3185

\* The mean difference is significant at the .05 level.

**Oneway**

**ANOVA**

**Mortality Female Tested**

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.505	5	1.301	2.854	.063
Within Groups	5.470	12	.456		
Total	11.975	17			

**Post Hoc Tests**

**Multiple Comparisons**

Dependent Variable: Female Tested  
LSD

(I) Grade of net's washings	(J) Grade of the net's washings	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 wash	6 washings	.0000	.5513	1.000	-1.2011	1.2011
	12 washings	-.3367	.5513	.553	-1.5377	.8644
	18 washings	-1.0233	.5513	.088	-2.2244	.1777
	21 washings	-1.6333*	.5513	.012	-2.8344	-.4323
	24 washings	-.9967	.5513	.096	-2.1977	.2044
6 washings	6 washings	.0000	.5513	1.000	-1.2011	1.2011
	12 washings	-.3367	.5513	.553	-1.5377	.8644
	18 washings	-1.0233	.5513	.088	-2.2244	.1777
	21 washings	-1.6333*	.5513	.012	-2.8344	-.4323
	24 washings	-.9967	.5513	.096	-2.1977	.2044
12 washings	6 washings	.3367	.5513	.553	-.8644	1.5377
	12 washings	.3367	.5513	.553	-.8644	1.5377
	18 washings	-.6867	.5513	.237	-1.8877	.5144
	21 washings	-1.2967*	.5513	.037	-2.4977	-9.5586E-02
	24 washings	-.6600	.5513	.254	-1.8611	.5411
18 washings	6 washings	1.0233	.5513	.088	-.1777	2.2244
	12 washings	1.0233	.5513	.088	-.1777	2.2244
	18 washings	.6867	.5513	.237	-.5144	1.8877
	21 washings	-.6100	.5513	.290	-1.8111	.5911
	24 washings	2.667E-02	.5513	.962	-1.1744	1.2277
21 washings	6 washings	1.6333*	.5513	.012	.4323	2.8344
	12 washings	1.6333*	.5513	.012	.4323	2.8344
	18 washings	1.2967*	.5513	.037	9.559E-02	1.8877
	21 washings	.6100	.5513	.290	-.5911	.5911
	24 washings	.6367	.5513	.271	-.5644	1.2277
24 washings	6 washings	.9967	.5513	.096	-.2044	2.1977
	12 washings	.9967	.5513	.096	-.2044	2.1977
	18 washings	.6600	.5513	.254	-.5411	1.8611
	21 washings	-2.6667E-02	.5513	.962	-1.2277	1.1744
	24 washings	-.6367	.5513	.271	-1.8377	.5644

\* The mean difference is significant at the .05 level.