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The Short-Term Toxicity of Two Toxicants to Artemia Nauplii

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Abstract: In this study, the short-term toxicities of sodium lauryl sulphate (an anionic detergent) and potassium dichromate on instar I and mixed instar II-III nauplii populations of *Artemia* were investigated.

The comparisons between our results and the ARC-test (Artemia Reference Center) protocol show our experiments to be acceptable for acute toxicity standardization.
Key Words: Artemia nauplii, anionic detergent (SLS), toxicity.

Artemia Naupliusları Üzerine İki Toksikantın Kısa Süreli Toksisitesi

Özet: Bu çalışmada, Artemia nauplius I ve karışık nauplius II-III populasyonları üzerine sodyum lauril sülfat (anyonik deterjan) ve potasyum dikromat'ın kısa süreli toksisitesi araştırılmıştır.

ARC-test (Artemia Reference Center) protokolu ile sonuçlarımızın karşılaştırılması bu denemenin de standart olarak kabul edilebileceğini göstermiştir.
Anahtar Sözcükler: Artemia naupliusları, anyonik deterjan (SLS), toksisite.

Introduction

Over the last two decades, a vast number of short-term aquatic toxicity tests have been developed for a wide range of aquatic organisms occupying different trophic levels, with the purpose of identifying chemicals with adverse effects in aquatic ecosystems.

The existing literature (1) shows that there is no uniformity in the methodologies nor in the criteria used to assess the dose-effect relationship.

The standardization of toxicity tests on aquatic organisms for detecting the impact of toxicants on freshwater or marine ecosystems is an urgent necessity. Scientists propose that the brine shrimp (Artemia) is a very suitable candidate for the development of a standard bioassay for worldwide utilization (2). A number of papers have been published on the toxic effects of various chemicals and toxicants on brine shrimp (*Artemia*) (3-8) *Artemia* cysts, nauplii and adults were used in these bioassays.

The majority of studies on *Artemia* nauplii deal with the acute and chronic toxicity of chemical compounds and abiotic factors on the early larval stages of *Artemia* (1, 2, 7, 9-15).Several similar studies have been done on adult brine shrimp (16-18). Data obtained from these studies indicate that adult *Artemia* are very well suited for long-term chronic bioassays.

In the present study, we investigated the short-term toxicity of two toxicants on *Artemia* nauplii using the standardized ARC-test developed by Artemia Reference Center at the State University of Ghent in Belgium.

Material and Method

Hatching of the cysts and collection of the nauplii

A homogenous population of the instar II-III and instar I naupalii hatched from cysts of a well-defined *Artemia* strain (San Francisco Bay, batch number 10-131-14) was used.

For each test approximately 100mg of cysts were incubated in 100ml seawater (35%0) in a cylindroconical tube at a temperature of 25±1°C and with lateral illumination by a light tube (500-1000 lux).

All cysts and hatching nauplii were kept in continuous suspension by gentle aeration from the bottom of the hatching device. In order to obtain a population consisting of first instar naupalii only, the hatched larvae were harvested after 18hr. One half of the population was used immediately for the test on instar I larvae. The other half was incubated for 24 hr at 25°C within an Erlenmeyer flask (200 ml) in gently aerated seawater. At the end of this period, more than 99% of the naupalii had molted into the instar II or, in some cases, the instar III stage. The larvae were poured into a large petri dish for subsequent manual distribution to the test petri dishes.

Preparation of the test

Prior to use, the seawater was filtered through a 1μm mesh filter under vacuum and then aerated. After aeration, the water was checked for a pH value of 7.5±0.5 and a minimum oxygen content of 90% saturation. This seawater was stored for no more than two weeks.

All experiments were carried out in glass petri dishes (diameter 60mm; height 12mm). Ten nauplii were transferred to each petri dish with a Pasteur pipette, which carried over less than 0.05ml of seawater into each dish. The dishes were then filled with 10ml of the respective concentrations of the toxicant (acclimated at 25°C), closed and incubated in darkness at a temperature of 25±1°C, for the respective test periods (6 and 24 hr).

After 6 or 24 hr, the number of dead larvae in each petri dish was determined. The nauplii were considered dead if no movement of the appendages was observed within 10 seconds. Immediately after counting, the oxygen concentration was measured in the petri dish with the lowest concentration of toxicant that induced 100% mortality.

Sodium laurly sulphate[1] (SLS) and potassium dichromate[2] (PDK) were chosen as toxicants.

For the preparation of 100mg/l SLS stock solution, it is recommended to dissolve the chemical at 25°C and to use a magnetic stirrer, since the product does not dissolve quickly. The stock solutions of the two toxicants were stored for no more than 48 hr.

All bioassays were carried out in two sections: preliminary tests and definitive tests. The concentrations of the toxicant to be tested were chosen from a logarithmic scale (19). For each concentration, including the control, three replicates were used. The larvae were not fed during the bioassay.

Two methods of calculation were used for the determination of the mean LC_{50} values (6 and 24 hr) of the toxicants. The LC_{50}, the 95% confidence limits and the slope function were calculated according to Litchfield and Wilcoxon (20). We also used Bliss' method (21) for the statistical comparison of the LC_{50} values obtained.

Results

Toxicities of the Polluants

The toxic effects of SLS and PDK within certain test periods (6 and 24 hr) on instar I and instar II-III larvae of *Artemia* were experimentally observed, and it was attempted to establish the effect limits.

Toxic effects of SLS on instar I nauplii

Six hours after the exposure of instar I to SLS, the effective mean LC_{50} value was observed to be between 75 and 87mg/l.

The linear mortality curve is given in Figure 1, according to Bliss' method, together with the related equation. The mean LC_{50} value of 74.45 mg/l for 6 hour is obtained with this equation (Table 1).

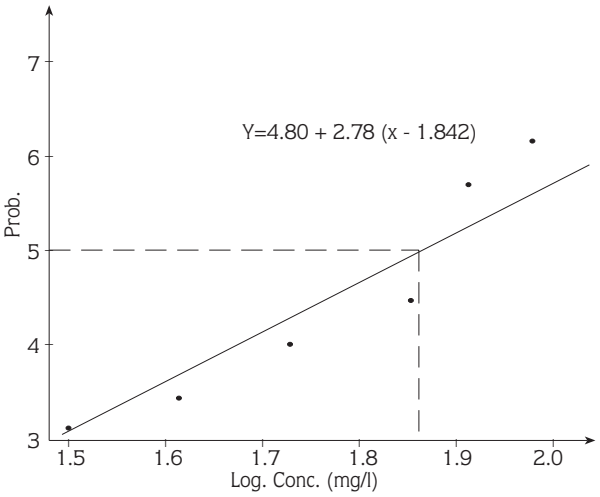

Figure 1. Mortality curve calculated by Bliss' method for 6hr with SLS on instar I larvae.

The mortality curve was computed from the mean LC_{50} of the replicates by the Litchfield-Wilcoxon method (Fig. 2).

In Table 1, the mean LC_{50} value, its 95% confidence limits, slope function (S) and factor (f) are summarized.

* [1] reference chemical (anionic detergent)
[2] unknown chemical

Bliss Method	Litchfield Method	
LC_{10} 41.45 ± 0.009 mg/l	LC_{16} 52 mg/l	
LC_{50} 74.45 ± 0.002 mg/l	LC_{50} 79.5 mg/l	
LC_{90} 117.24 ± 0.009 mg/l	LC_{84} 95 mg/l	S=1.36
		f=1.09
Graphic Method		Upp. Lim.: 86.65 mg/l
LC_{50} 75.85 mg/l		Low. Lim.: 72.93 mg/l

Table 1. Results of 6hr test obtained by different methods with SLS on instar I larvae.

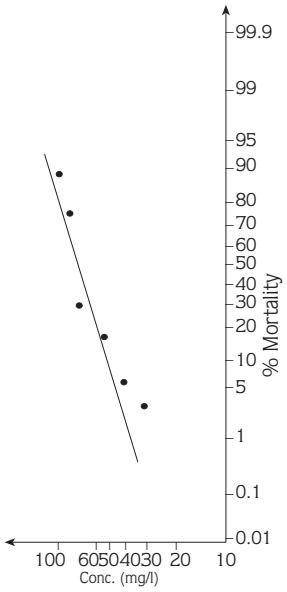

Figure 2. Mortality curve calculated by Litchfield and Wilcoxon's method for 6hr with SLS on instar I larvae.

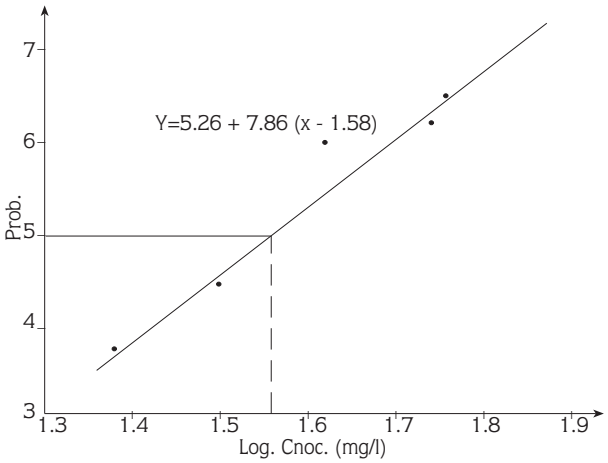

Figure 3. Mortality curve calculated by Litchfield and Wilcoxon's method for 24hr with SLS on instar I larvae.

Test duration in the second group of experiments was 24 hr. In the second toxicity test, larvae were affected by the lower concentrations of SLS. In all concentrations with values above 56 mg/l, total mortality was observed.

At the end of this test period, the mean 24 hr LC_{50} value of the toxicant was observed to be between 32 and 42 mg/l.

The mortality curve obtained according to the Bliss method is shown Figure 3. It is clear from the results (Table 2) that the mean LC_{50} value obtained according to the Bliss method and that obtained by the graphic method derived from it are very close.

The results of the data obtained by the Litchfield-Wilcoxon method are summarized in Table 2. Figure 4 shows the mortality curve calculated from the mean 24 hr LC_{50}'s for three replicates.

Toxic effects of SLS on instar II-III nauplii

According to the bioassay results, the mean LC_{50} value was observed to be between 42 and 56mg/l 6 hr after exposure to SLS on instar II-III.

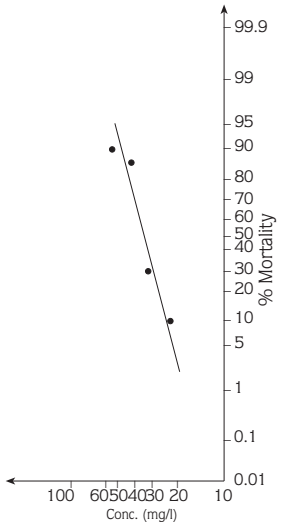

Figure 4. Mortality curve calculated by Litchfield and Wilcoxon's method for 24hr with SLS on instar I larvae.

The mortality curve is given in Figure 5, according to the Bliss method, along with the related equation. The mean LC_{50} value of 43.52mg/l for 6 hours was obtained with this equation Table 3. This value differs significantly from the value found for the 6-hr test with instar I larvae.

The value (3.86) found by the X^2 test within the Bliss method is less than P value 9.48 (at the $P<0.05$ level) Therefore, the *Artemia* instar II-III population can be considered homogenous. The ability to use the X^2 test according to Bliss's method is an additional advantage.

Following by the Litchfield-Wilcoxon method. The mortality curve calculated from the mean LC_{50} of three replicates by the Litchfield-Wilcoxon method is shown in Figure 6. The mean LC_{50} value, its 95% confidence limits, slope fucntion and factor are summarized in Table 3.

During the 24-hr experiments. For example, 10%, 30% and 96% mortality was observed in 5.6 mg/l, 10 mg/l and 32 mg/l concentrations, respectively instar II-III larvae were affected by the lowest concentrations, of SLS. The mean LC_{50} was between 13.5 and 18mg/l in the 24-hr experiment.

Bliss Method	Litchfield Method	
LC_{10} 21.72 ± 0.010 mg/l	LC_{16} 27 mg/l	
LC_{50} 35.14 ± 0.002 mg/l	LC_{50} 35 mg/l	
LC_{90} 51.13 ± 0.006 mg/l	LC_{84} 44 mg/l	S=1.28
		f=1.09
Graphic Method		Upp. Lim.: 38.15 mg/l
LC_{50} 36.22 mg/l		Low. Lim.: 32.11 mg/l

Table 2. Results of 24-hr test obtained by different methods with SLS on instar I larvae.

Bliss Method	Litchfield Method	
LC_{10} 29.10 ± 0.004 mg/l	LC_{16} 26 mg/l	
LC_{50} 43.52 ± 0.001 mg/l	LC_{50} 43 mg/l	
LC_{90} 59.56 ± 0.003 mg/l	LC_{84} 69 mg/l	S=1.63
		f=1.13
Graphic Method		Upp. Lim.: 48.59 mg/l
LC_{50} 43.65 mg/l		Low. Lim.: 38.05 mg/l

Table 3. Results of 6-hr test obtained by different methods with SLS on instar II-III larvae.

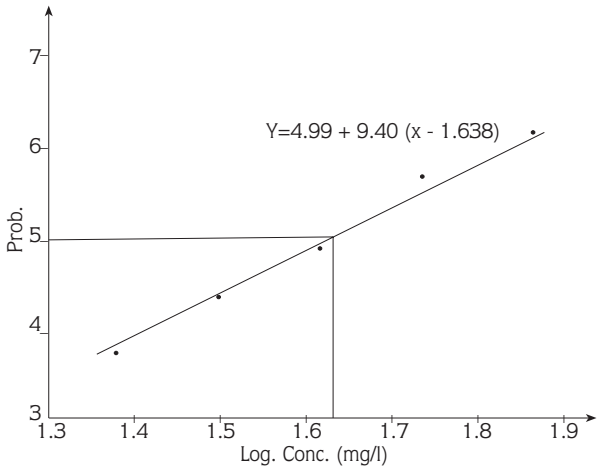

Figure 5. Mortality curve calculated by Bliss' method for 6hr with SLS on instar II-III larvae.

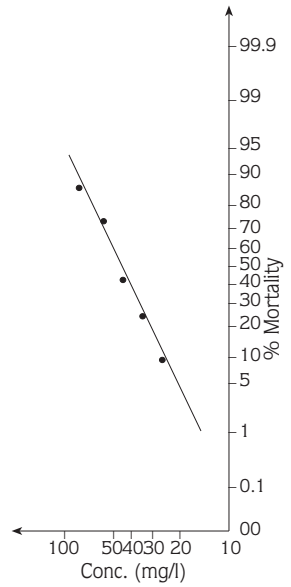

Figure 6. Mortality curve calculated by Litchfield and Wilcoxon's method for 6hr with SLS on instar II-III larvae.

The equation of the mortality curve found by Bliss's method is given in Figure 7. The mean LC_{50} value calculated using this equation is 13.84mg/l. The upper and lower limits between which the lethal concentration lies and their variances are given in Table 4. The mortality curve calculated by the Litchfield-Wilcoxon method is shown in Figure 8.

A comparison of the mean LO_{50} values in Table 4 reveals that the values calculated with different statistical methods are very close and support our experimental observations.

Toxic effects of PDK on instar II-III nauplii

We decided to compare toxic effects of PDK with SLS because this bioassay is recommended for *Artemia* by several authors (2, 10, 11, 22). The bioassay was carried out with instar II-III nauplii. The test duration was 24-hr.

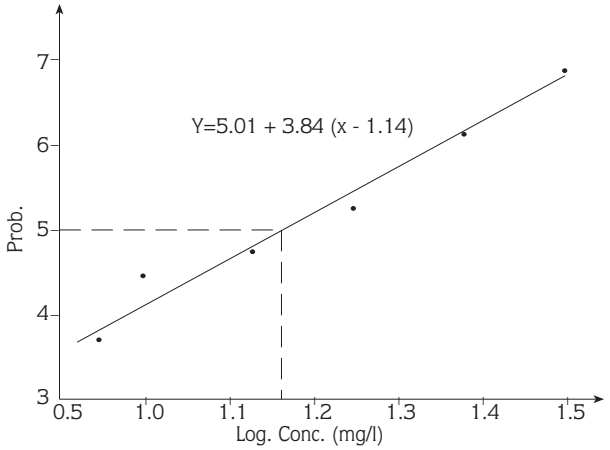

Figure 7. Mortality curve calculated by Bliss' method for 24hr with SLS on instar II-III larvae.

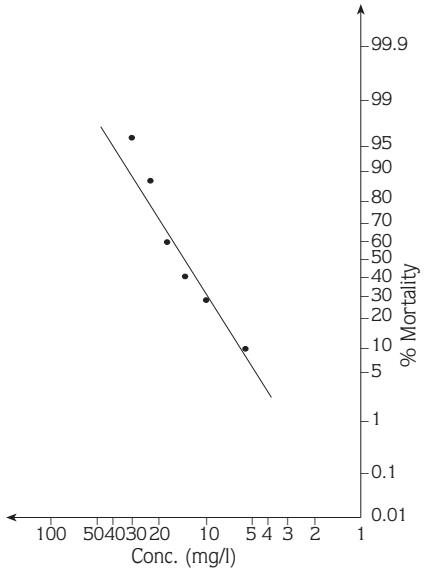

Figure 8. Mortality curve calculated by Litchfield and Wilcoxon's method for 24hr with SLS on instar II-III larvae.

According to the definitive test, 24-hr after the exposure of instar II-III to PDK, the mean LC_{50} value was observed to be between 32 and 42mg/l.

The mortality curve obtained according to the Bliss method is given in Figure 9, together with the related equation. The mean LC_{50} value and other lethal concentrations such as LC_{90}, LC_{10} are shown in Table 5. The mortality curve obtained by the Litchfield-Wilcoxon method is shown in Figure 10. The results are summarized in Table 5. From the LC_{50} values it is clear that *Artemia* larvae are more sensitive to sodium lauryl sulphate than to potassium dichromate (in Table 4 and 5).

Bliss Method	Litchfield Method	
LC_{10} 5.12 ± 0.021 mg/l	LC_{16} 7.4 mg/l	
LC_{50} 13.84 ± 0.005 mg/l	LC_{50} 14.5 mg/l	
LC_{90} 29.56 ± 0.016 mg/l	LC_{84} 27 mg/l	S=1.91
		f=1.18
Graphic Method		Upp. Lim.: 17.11 mg/l
LC_{50} 14.48 mg/l		Low. Lim.: 12.29 mg/l

Table 4. Results of 24-hr test obtained by different methods with SLS on instar II-III larvae.

Bliss Method	Litchfield Method	
LC_{10} 11.34 ± 0.031 mg/l	LC_{16} 17.5 mg/l	
LC_{50} 32.84 ± 0.007 mg/l	LC_{50} 34 mg/l	
LC_{90} 75.17 ± 0.017 mg/l	LC_{84} 60 mg/l	S=1.85
		f=1.16
Graphic Method		Upp. Lim.: 39.44 mg/l
LC_{50} 33.49 mg/l		Low. Lim.: 29.31 mg/l

Table 5. Results of 24-hr test obtained by different methods with PDK on instar II-III larvae.

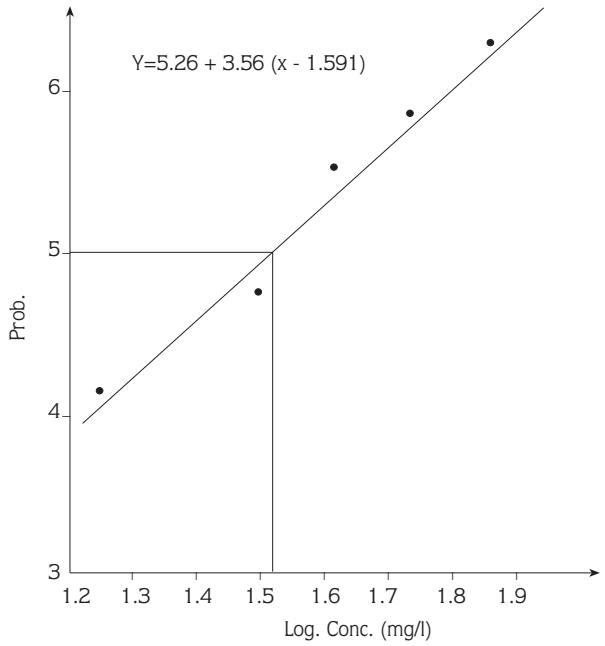

Figure 9. Mortality curve calculated by Bliss' method for 24hr with PDK on instar II-III larvae.

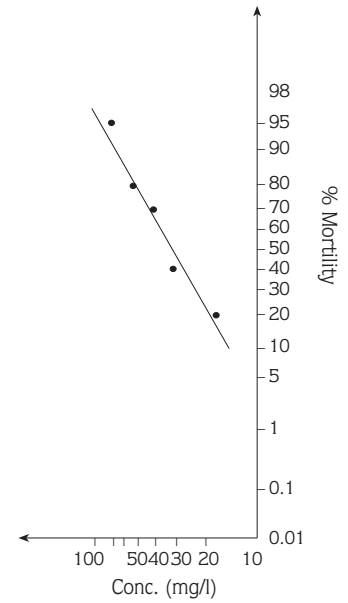

Figure 10. Mortality curve calculated by Litchfield and Wilcoxon's method for 24hr with PDK on instar II-III larvae.

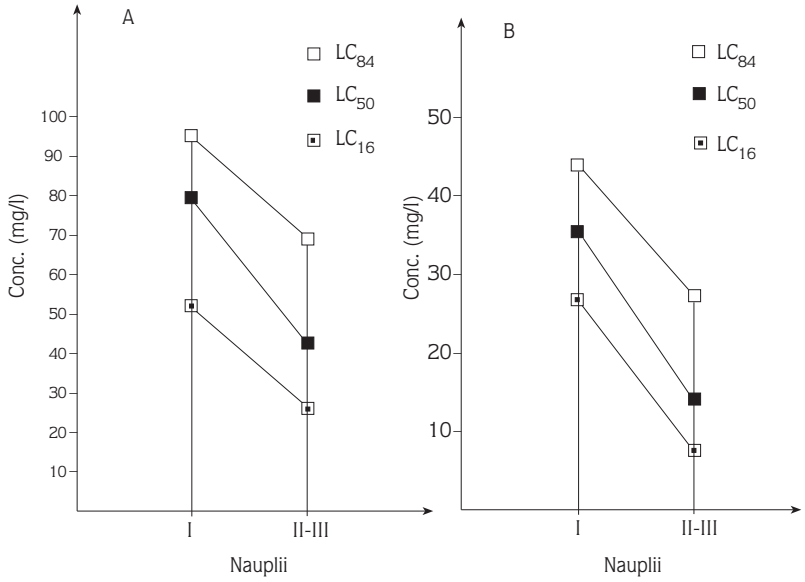

Figure 11. The comparison of the sensitivity of the Artemia nauplii exposed (A: for 6hr, B: for 24hr) to the SLS.

Comparison of the Sensitivities of Artemia Nauplii

The results of our experimental observations reveal that there is a marked difference in sensitivity between instar I and instar II-III larvae exposed to the detergent. This is because *Artemia* nauplii have different morphological and anatomical features.

In the 6-hr toxicity test with instar I nauplii, SLS acted within a broad range of concentrations (52-95 mg/l) and at high concentrations. In contrast, it was found to affect instar II-III nauplii within a narrower range of concentrations (26-69 mg/l) and at lower concentrations (Fig. 11A).

Similar results were found in the 24-hr toxicity tests, i.e., the mean 24-hr LC_{50} value for instar I was 35 mg/l the mean LC_{50} for instar II-III was 14mg/l. These results support the findings obtained by Bliss' method (Fig. 11B).

The results celarly show that instar II-III larvae are significantly more sensitive to the detergent than instar I larvae.

Discussion

The toxic effectcs of SLS and PDK within certain test periods (6 and 24-hr) on instar I and instar II-III larvae of *Artemia* were experimentally observed and their effect limits were established.

According to the bioassay results, SLS acts within a broad range of concentrations with instar I nauplii. The mean 6 hr-LC_{50} value was 79.5mg/l, and the 95% confidence limits of LC_{50} s were 72.83-86.65 mg/l. The mean 6-hr LC_{50} value obtained by a similar method Vanhaecke et al. (2) was also 77.4 ppm. Similar results were found in the 24-hr test.

During both the 6- and 24-hr tests, instar II-III larvae were affected by a lower concentration of SLS than instar I larvae were. We observed total mortality at concentrations of higher than 75mg/l. (6hr) and 32mg/l. (24hr). The results obtained by Vanhaecke et al. (2) are very cloes (6 hr-LC_{50} 44.2 ppm. and 24 hr-LC_{50} 17.8 ppm.) and support our data.

From these results it is clear that instar II-III larvae are significantly more sensitive than instar I larvae. These results confirm the findings of the previous researchers (2, 9, 12, 13).

This situation may be explained by the fact taht in fershly hatched nauplii the epithelium of the digestive tract is not in contact with the external medium, as explained by some authors (13). The studies on the larval stages of development of *Artemia* (23-25) show that the permeability of the digestive tube at the early larval stage is not complete. Castritsi-Catharios et al. (15) observed the effect of the dispersant on instar II-III nauplii by electron microscopy at the epithelium layer of mid-gut, which in particular shows a deterioration of microvilli.

According to the 24-hr bioassay results, instar II-III larvae were affected by a higher concentration of PDK. Similar results have been obtained by other authors (2, 13).

On the basis of these results it is clear that *Artemia* larvae are more resistant to PDK than to SLS.

Conclusion

From the literature it is clear that the major advantage of *Artemia* for toxicity and aquaculture studies is the overall availability of the dry cysts.

In this paper we have attempted to determine the short-term toxicity of two chemicals on *Artemia* nauplii. Vanhaecke et al. (12) emphasize that the interval in which the LC_{50} of sodium laurly sulphate must be situated ranged between 13.3 and 19.9 ppm.

Our mean LC_{50} value is within this range as well.

Comparisons between our results and the ARC-test (Artemia Reference Center) protocol show our experiments to be acceptable for standardization on acute toxicity.

Acknowledgement

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