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POSTLARVAE

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# ACUTE TOXICITY OF DRILLING MUDS ON *LITOPENAEUS* *VANNAMEI* (BOONE, 1931) POSTLARVAE

## *TOXICIDAD AGUDA DE LODOS DE PERFORACIÓN EN POSTLARVAS DE LITOPENAEUS VANNAMEI (BOONE, 1931)*

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### ABSTRACT

**A**cute toxicity of six drilling muds (water based and synthetic based) was evaluated using *Litopenaeus vannamei* postlarvae to establish the 96 h median lethal concentration (LC<sub>50</sub>). The Suspended Particulate Phase (SPP) of each drilling mud was used as test material. Each mud SPP was evaluated using five test concentrations, and four replicates of each were used including the control. LC<sub>50</sub> values obtained ranged from 4224 ppm to 26635 ppm for Water Based Mud (WBM) and from 40781 ppm to 308248 ppm for Synthetic Based Mud (SBM). The test protocol for acute toxicity for this species was validated through a control chart with the reference toxicant potassium dichromate. *Litopenaeus vannamei* clearly showed the required conditions to be used in acute toxicity tests. This research is pioneer in Colombia to determine the effects of drilling muds using a local marine organism.

**Keywords:** Drilling muds, Toxicity, Marine organisms, Environmental impact, Offshore.

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## RESUMEN

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**L**a toxicidad aguda de seis lodos de perforación (base agua y base sintético) fue evaluada utilizando postlarvas de *Litopenaeus vannamei* con la finalidad de establecer la concentración letal media a 96 h (CL<sub>50</sub>). La Fase Suspendida Particulada (FSP) de cada lodo fue usada como material de prueba. Cada FSP de los lodos fue evaluada usando cinco concentraciones de prueba con cuatro réplicas de cada una, incluido el control. Los valores de CL<sub>50</sub> obtenidos de la FSP de los Lodos Base Agua (LBA) se encontraron entre 4224 ppm y 26635 ppm y entre 40781 ppm y 308248 ppm para los Lodos Base Sintética (LBS). El protocolo de ensayo para toxicidad aguda con esta especie se validó mediante una carta control con el tóxico de referencia (dicromato de potasio). *Litopenaeus vannamei* mostró tener las condiciones requeridas para ser usado en pruebas de toxicidad aguda. Este trabajo es pionero en Colombia para evaluar los efectos de los lodos de perforación con un organismo marino local.

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**Palabras clave:** Lodos de perforación, Toxicidad, Organismos marinos, Impacto ambiental, Costa afuera.

## RESUMO

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**A** toxicidade aguda de seis lamas de perfuração (base água e base sintética) foi avaliada utilizando pós-larvas de *Litopenaeus vannamei* com a finalidade de estabelecer a concentração letal média a 96 h (CL<sub>50</sub>). A Fase Suspensa Particulada (FSP) de cada lama foi usada como material de prova. Cada FSP das lamas foi avaliada usando cinco concentrações de prova com quatro réplicas de cada uma, incluindo o controle. Os valores de CL<sub>50</sub> obtidos da FSP das Lamas Base Água (LBA) estavam entre 4224 ppm e 26635 ppm e entre 40781 ppm e 308248 ppm para as Lamas Base Sintética (LBS). O protocolo de ensaio para toxicidade aguda com esta espécie foi validada mediante uma carta controle com o tóxico de referência (dicromato de potássio). *Litopenaeus vannamei* mostrou ter as condições necessárias para ser usado em provas de toxicidade aguda. Este trabalho é pioneiro na Colômbia para avaliar os efeitos das lamas de perfuração com um organismo marinho local.

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**Palavras-chave:** Lamas de perfuração, Toxicidade, Organismos marinhos, Impacto ambiental, Offshore.

## 1. INTRODUCTION

Exploration of offshore oil and gas is conducted on a worldwide scale and long term developments can cause physical, chemical and biological alteration in marine environments (Neff, 2005). Well drilling is one of the main activities in different phases of hydrocarbon development. Drilling muds used for drilling are mixtures of natural and synthetic compounds that have different functions such as: cool down and lubricate the drilling string and drilling bits, remove cuts from downhole carrying them to the surface and control pressure problems, among others (Fink, 2011). Three types of drilling muds are used, including Water Based Muds (WBM), which continuous or liquid phase is seawater and two types of non-aqueous muds: Oil Based Mud (OBM) and Synthetic Based Muds (SBM). The WBM are more commonly used in the main areas of offshore oil drilling while OBM is no longer used. During drilling activities, muds and cuttings wastes are generated in considerably amounts and their discharge to the ocean is regulated in many countries, mainly due to concerns on the impacts of solids and toxicity of chemical waste in the water column and benthic ecosystems (Neff, 2008).

The assessment of potential impacts to the marine environment is established through acute toxicity, chronic toxicity and bioaccumulation studies (Melton *et al.*, 2000). A wide variety of organisms have been used to evaluate the acute toxicity of drilling muds. In 1983, the National Research Council (NRC) published a report making an assessment of fates and effects of drilling fluids in marine environment and an evaluation was performed -based on secondary information- of 400 assays with 72 WBM conducting on 62 different marine species from seas of the United States of America (USA). The organisms used included fish, crustaceans, molluscs, polychaeta and echinoderms. Crustaceans, specifically copepods, mysids and shrimp in early life stages, demonstrated to be more sensible than other taxa (NRC, 1983). In 1985, the Environmental Protection Agency of the United States (EPA) determined the use of *Mysidopsis bahia* (Crustacea) as the organism to carry out acute toxicity tests, establishing a 96 h LC<sub>50</sub> value of >30000 ppm of the SPP to allow discharges of WBM into marine waters of the United States of America (USA) (EPA, 1993; Code of Federal Regulations, 2012). In Brazil, *Mysidopsis*

*juniae* is used for testing and the acute toxicity value required for mud discharges is similar to the one in the USA. (Cupello, Marroquin, Franca & Rabke, 2010).

The objective of this study was to validate a protocol for the evaluation of acute toxicity using *Litopenaeus vannamei* and to test with it six drilling muds (3 WBM and 3 SBM).

The white shrimp (*L. vannamei*), is distributed in the Pacific coast of America, from Southern México to Northern Perú (Luvesuto, Domingues de Freitas & Galleti, 2007; Rivera-Velásquez, Salgado-Ugarte, Soto & Naranjo, 2010). It is an epibenthic species that, as an adult, lives and reproduces in ocean waters, while postlarvae migrates to the coast spending juvenile and pre-adult phase in estuaries (Campos, Navas, Bermúdez & Cruz, 2005; Valles-Jiménez, Cruz & Pérez-Enríquez, 2005). It is also an important catch group and represents a high percentage of industrial and handcrafted fishing in the Colombian Pacific coast (SIPA, 2009). *L. vannamei* have been introduced successfully in the colombian Caribbean coast in acuculture facilities with comercial propourses. Juvenile organisms or postlarvae of these species have been used to evaluate pesticides, oil dispersants and mainly toxicity of metals; characteristics of assays are shown in Table 1. Due to the availability of organisms from hatchery production under controlled conditions, it becomes an important test species for Colombia. Based on existing protocols from EPA (2002), the *Asociacao Brasileira de Normas Técnicas* - ABNT (2005) and related literature on drilling muds and acute toxicity tests with marine organisms, a protocol for evaluation of acute toxicity of drilling muds on *L. vannamei* was generated.

## 2. MATERIALS AND METHODS

### Sea Water

Natural seawater was used for organism maintenance, muds preparation and test dilutions. Water was obtained from Gaira Bay (Santa Marta, Colombia), prefiltered through four terraces of gravel, a series of 20 and 1 µ filters, activated charcoal and finally sterilized using UV light.

**Table 1.** Conditions for acute tests at 96 h on *L. vannamei*.

Evaluated substance	Number of organisms per replicate	Larval stage	Food supply	Aeration	Test flasks	Number of concentrations	Number of replicates	Test type	LC <sub>50</sub> - 96 h (ppm)	Author
Oil dispersant	5	Postlarvae (PL 5)	Yes	-	Glass containers (125 mL)	5 to 8	-	Static	4 - 10*	Villamar, 1990
Cd Hg Pb	10	Postlarvae (PL 12)	Yes	Yes	Beaker (600 mL)	-	3	SR (24 h)	2.49* 1.23* 134*	Frías-Espericueta, Voltolina & Osuna-López, 2001
Cu Zn Mn	10	Postlarvae (PL 12)	-	Yes	Beaker (600 mL)	-	-	SR (24 h)	- 2.08* 130*	Frías-Espericueta, Voltolina & Osuna-López, 2003
Cd Zn	10	Postlarvae	-	-	Beaker (1L)	7 5	3	SR (24 h)	1.07* 1.35*	Wu & Chen, 2004
Methamidophos	10	Juvenile	-	Yes	Aquariums (7 L)	6	-	-	1.46*	García-de la Parra <i>et al.</i> , 2006
Cu	-	Juvenile	-	Yes	-	5	-	SR (24 h)	35.12*	Frías-Espericueta <i>et al.</i> , 2008
B (20‰) B (3‰)	20	Juvenile	Yes	-	-	6	3	SR (24 h)	80.06* 25.05*	Li <i>et al.</i> , 2008

\*The original values are expressed in mg/L.

SR: static renewal.

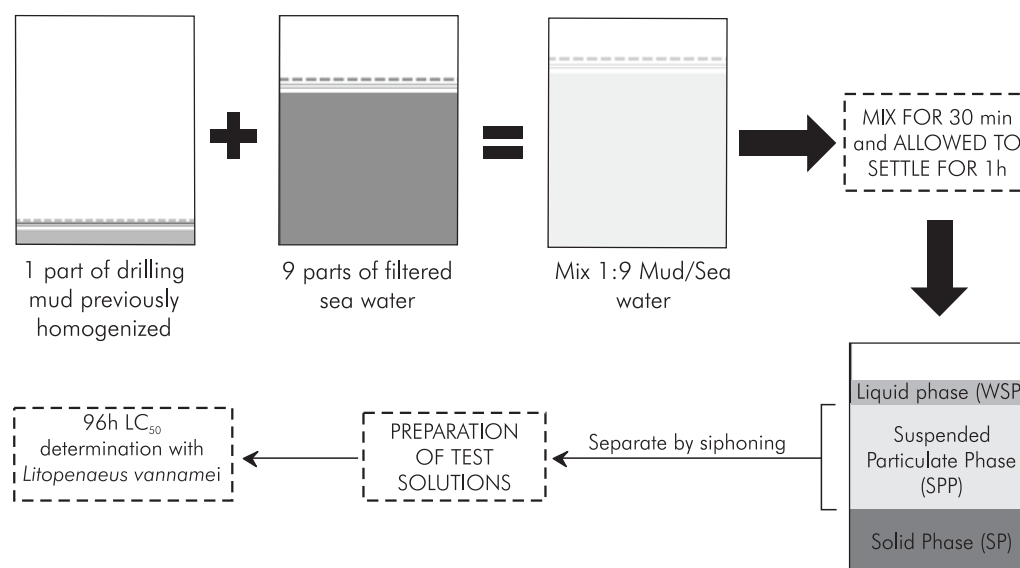
### Organisms Supply and Maintenance

*L. vannamei* postlarvae 20 d (PL10) to 30 d (PL15) were supplied by the *Centro de Investigación de la Acuicultura en Colombia* (CENIACUA) located in Punta Canoa, near Cartagena. Transportation was carried by land in special plastic bags with 20 L of marine water with oxygen saturation and controlled temperature and salinity conditions. Once in the laboratory, organisms were placed in 50 L aquarium with seawater salinity between 33 and 37, constant aeration, 12 h light - 12 h dark photoperiod, 30% daily water renewal and food supply (PL-400 25%, 50% Nicovita and 25% pulverized Artemia) three times a day. Before their use for toxicity assays, organisms were in a period of acclimation between four to seven days. Considerations for their use were those established by the United States Environmental Protection Agency (EPA), which indicate that mortality during acclimation period cannot be more of 10% (EPA, 2002).

### Drilling Mud Preparation

Muds were prepared at the Oceanography and Chemical Laboratory of the *Universidad Jorge Tadeo Lozano de Bogotá*, Santa Marta, by staff from the companies providing the muds, guaranteeing that preparation is similar to the one performed for drilling use. Samples were identified as E1, E2 and E3 (Companies) with the added letter W to refer WBM or the letter S in case of SBM. Conditions for mud storage and obtaining of Suspended Particulate Phase (SPP) for bioassays were based on Duke *et al.* (1984), Jones *et al.* (1986) and EPA (1993) recommendations.

The SPP was obtained previously by homogenizing the mud for 10 minutes with an electric mixer and then mixing one part of mud with nine parts of filtered seawater (Figure 1). Then, slurry was constantly mixed for 30 min and then allowed to settle for 1 h. To separate the SPP, liquid slightly below



**Figure 1.** Suspended Particulate Phase (SPP) obtainment from drilling muds.

the surface and above the sediment or solid phase was siphoned and stored in a suitable container. This fluid represented the 100 % SPP for the various test concentrations preparation.

### Acute Toxicity Tests

Static renewal tests were performed to determine the 96 h median lethal concentration ( $LC_{50}$ ). Range finding tests were conducted with serial decimal dilutions from 100% SPP to establish concentrations in the definitive tests; concentrations for definitive test were obtained mainly using a 0.5 factor from the minimum concentration where all organisms die in range finding test. During testing, five concentrations and a negative control with seawater were used, four replicates for each treatment and a total of 20 organisms placed randomly (five per replicate) in 1 L plastics containers with 500 ml of test solution. Shrimps were fed two times a day and total renewal of solutions was done at 48 h (Table 2). Physicochemical parameters (PHC), were recorded (pH, salinity, temperature and dissolved oxygen) at least in one of the replicates of each concentration and control at the beginning and end of test. Mortality was recorded every 24 h, considering dead organisms those with lack of motility in the bottom of containers. As a test acceptability criterion, mortality in the control must not exceed 10%.

Definitive testing concentrations used for each mud were: E1W (20000, 25000, 30000, 35000 and 40000

ppm), E2W (625, 1250, 2500, 5000 and 10000 ppm), E3W (6250, 12500, 25000, 50000 and 100000 ppm); E1S (6250, 12500, 25000, 50000 and 100000 ppm); E2S (5000, 10000, 50000, 100000 and 500000 ppm) and E3S (37500, 75000, 150000, 300000 and 400000 ppm).

**Table 2.** Experimental conditions for the acute test with *L. vannamei*.

Test type	Static
Time of exposure	96 h
Food supply	Twice daily
Larval stage	Postlarvae (PI 10 - PI 15)
Temperature	25 - 27 °C
Salinity	33 - 37
Airation	No
Solution renewal	48 h
Dilution water	Filtered sea water
Photoperiod	12 h light / 12 h dark
Test flasks	Plastic (1 L)
Volume of sample test	500 ml
Number of concentrations	5 (plus control)
Number of replicates	4
Number of organisms per replicate	5
Final measured effect	Lethality; $LC_{50}$ 96 h
Test validation	Mortality in control $\leq$ 10%

### Statistical Analysis

Median lethal concentrations (96h LC<sub>50</sub>) with 95% confidence interval were determined using free access software obtained from EPA (V. 1.5 Probit Analysis Program or Trimmed Spearman Karber).

### Control Chart

Potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) was used as reference toxicant. Control chart was generated in order to record the sensitivity, reproducibility and stability of the biological response of *L. vannamei*. Tests were performed under the same test conditions used with drilling muds to validate the protocol according to the criteria of the EPA (2002) and EPS (1990).

General process followed for acute toxicity assays of drilling muds with *L. vannamei* in the present study is shown in Figure 2.

## 3. RESULTS AND DISCUSSION

### Mortality

During the first 48 h, mortality for E1W and E3W were higher in the second highest concentration (35000 and 50000 ppm respectively); after 48 h mortality of organisms increased due to the effects caused by drilling muds. The highest mortality at 96 h for evaluated muds were found in the higher concentrations and mortality found in controls did not exceed 10% (Table 3).

Mortalities for SBM were always higher at high concentrations during the time of test (100000, 500000 and 400000 ppm). E2S show no mortality at concentrations of 5000 and 10000 ppm, while E1S control presented 10% mortality (Table 4).

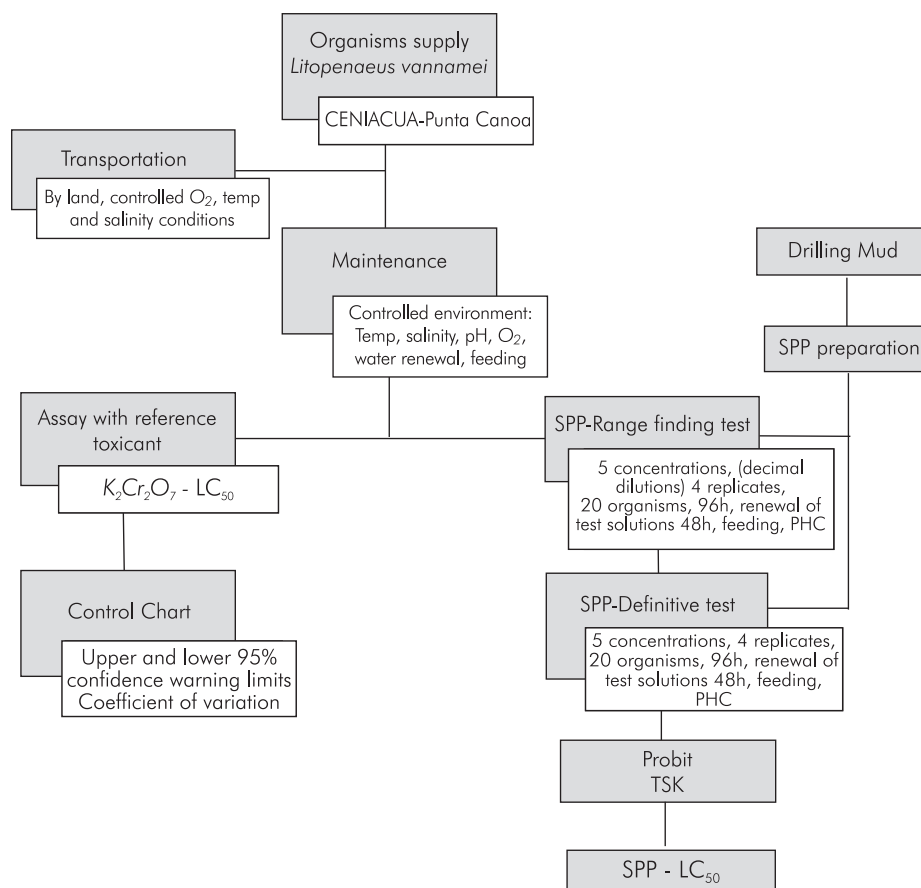


Figure 2. General procedure for an acute toxicity test of drilling mud with *L. vannamei* postlarvae.

**Median Lethal Concentration**

96 h acute toxicity values of drilling muds for *L. vannamei* with a 95% confidence interval are reported

(Table 5). For WBM, lower LC<sub>50</sub> was 4224 (E2W) and the highest was 26635 ppm in E3W.

**Table 3.** Percentage mortality of *L. vannamei* exposed to water based drilling muds.

Time of exposure (h)	E1W						E2W SPP (ppm)						E3W					
	0	20 000	25 000	30 000	35 000	40 000	0	625	1 250	2 500	5 000	10 000	0	6 250	12 500	25 000	50 000	100 000
24	0	20	30	5	45	25	0	0	0	0	0	0	0	0	0	30	45	35
48	0	20	30	15	45	40	0	0	0	0	15	40	0	0	0	30	45	35
72	0	45	40	50	75	85	10	5	25	30	55	90	5	0	0	50	90	95
96	0	45	40	55	75	85	10	5	25	30	55	90	5	0	0	50	90	95

SPP: solid particulate phase from drilling mud

**Table 4.** Percentage mortality of *L. vannamei* exposed to synthetic based drilling muds.

Time of exposure (h)	E1S						E2S SPP (ppm)						E3S					
	0	6 250	12 500	25 000	50 000	100 000	0	5 000	10 000	50 000	100 000	500 000	0	37 500	75 000	15 0000	300 000	400 000
24	5	10	0	5	0	20	0	0	0	0	0	45	0	0	0	5	10	30
48	10	10	0	5	0	20	0	0	0	0	0	45	0	0	5	5	25	30
72	10	10	5	10	40	95	0	0	0	25	10	65	0	0	5	5	40	45
96	10	10	5	20	70	100	0	0	0	25	10	65	0	0	5	40	45	55

SPP: solid particulate phase from drilling mud

**Table 5.** Median letal concentration (with 95% confidence limits) in *L. vannamei*.

	Water based drilling muds			Synthetic based drilling muds		
	E1W	E2W	E3W	E1S	E2S	E3S
LC <sub>50</sub> 96 h	24 841	4 224	26 635*	40 781	301 813	308 249
Lower limit	18 027	2 542	21 712	30 058	175 484	226 503
Upper limit	28 799	6 045	32 674	50 073	766 085	510 325

\*Calculated with Trimmed Spearman Karber analisis, all others with Probit analisis.



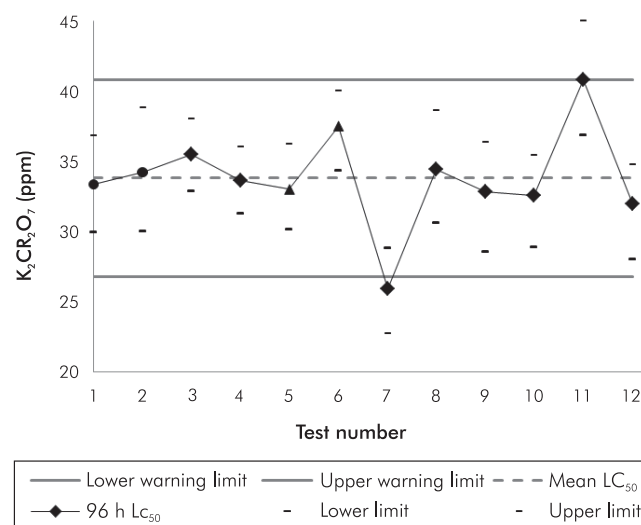
The lowest  $LC_{50}$  found in SBM was 40781 ppm for E1S and the highest was 308248 ppm for E3S. Synthetic muds analyzed reached the highest toxicity values above 40000 ppm of the SPP. The 96 h  $LC_{50}$  for WBM ranged between 4224 to 26635 ppm of the SPP. These results are within the range reported by other authors who have evaluated the toxicity of drilling muds with different species of marine organisms. In 1983, the report of the National Research Council found that almost 80% of the  $LC_{50}$  values of 400 muds assays with 62 species of marine organisms were above 10000 ppm (NRC, 1983; Neff, 2005). Evaluating the SPP of drilling muds Duke *et al.*, (1984), determined the toxicity of eight generic drilling fluids with *Mysidopsis bahia*, finding  $LC_{50}$  in the range of 27000 to > 1000000 ppm, being the lowest for the mud type KLC polymer. Duke and Parrish (1985), reported the results of an evaluation program of 11 drilling muds used in the Gulf of México finding a range of toxicity to mysids between 726 and 50000 ppm. The same muds were analyzed with shrimp *Paleomonetes pugio* and the range found was between 142 to >100000 ppm of whole mud. To a KLC-Polymer mud, Viega *et al.* (2001) reported an  $LC_{50}$  of 26400 ppm with Brazilian species *Mysidopsis juniae*. In Indonesia, five active drilling muds were evaluated with *Penaeus monodon*,  $LC_{50}$  values ranged from 30740 to 78271 ppm (Soegianto, Irawan & Affandi, 2008).

All drilling muds were considered nontoxic (Table 6), according to the acute aquatic toxicity classification of the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP, 2002).

**Table 6.** Revised classification for acute aquatic toxicity from GESAMP (2002).

Classification	Description	$LC_{50}$ (ppm)
0	No toxic	> 1000
1	Practically non toxic	> 100- ≤ 1000
2	Slightly toxic	> 10- ≤ 100
3	Moderately toxic	> 1- ≤ 10
4	Highly Toxic	> 0.1- ≤ 1
5	Very highly toxic	> 0.01- ≤ 0.1
6	Extremely toxic	≤ 0.01

According to EPA (2002), is the responsibility of the laboratory to demonstrate its ability to obtain accurate and consistent results with a reference toxicant to validate a method that will be used for regulatory purposes. This intralaboratory precision is expressed as the coefficient of variation (CV%) and should be determined by performing five or more tests with different groups of organisms under the same test conditions and data analysis, setting a CV% of ≤ 30% as the maximum allowable variability when there are no standards references (EPS, 1990; Silva, Torrejon, Bay-Shmith & Larrain, 2003). In the same way, a control chart should be prepared with at least 5 successive values of the reference toxicant to plot the average of all values and the upper and lower 95% confidence warning limits in a graphic. If two or more consecutive values fall outside the limits or seven consecutive results fall on the same side of the midline, procedures should be reviewed and should not be done any test until the proceedings are revalidated (EPA, 2002; ABNT, 2005). In the present study, we constructed the control chart with the reference toxicant, making 12 assays in parallel with drilling muds tests. The sensitivity of *L. vannamei* to  $K_2Cr_2O_7$  was determined and a 96 h  $LC_{50}$  average of 33.80 (± 3.51) ppm was obtained (Figure 3), setting a sensitivity range at 95% represented by the lower limit of 26.78 and upper limit of 40.81 ppm. The coefficient of variation obtained was 10.38 %, so the accuracy achieved was superior to the suggested (EPS, 1990).



**Figure 3.** Control chart 96 h acute test with potassium dichromate for *L. vannamei*.

#### 4. CONCLUSIONS

- A protocol for acute toxicity evaluation generated during this research was successfully validated through a control chart using  $K_2Cr_2O_7$  as reference toxicant. Precision and accuracy of the results showed the correct application of the methodology and stability in the sensibility of *L. vannamei*.
- All drilling muds were considered as non-toxic according to GESAMP classification for acute aquatic toxicity established for chemicals substances.
- *L. vannamei* meets the requirements to be used in acute toxicity tests since it is obtained from a continuous production laboratory, is easily maintained under laboratory conditions and has been used worldwide.
- In order to take part in the regulation of drilling fluids discharges in the marine environment, toxicity studies in the country must continue. It is important to extend the range of native species to work with in terms of lethal and sub lethal effects (e.g. reproduction, growth, physiological response and DNA damage). Since lethal effects showed that drilling muds are not acutely toxic, sublethal effects in lower concentrations of  $LC_{50}$  should be evaluated in the future.

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