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# Toxicity Evaluation of Common Drilling Fluid Additives and Applicability Comparison of Marine Toxicity Test Organisms

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#### Abstract

The use of drilling additives has rapidly increased with the growing complexity of the drilling process. The environmental hazards associated with drilling waste have drawn a great deal of attention. It is, therefore, necessary to select eco-friendly drilling fluid additives and screen for suitable bioindicators. In the present study, toxicity tests were conducted in accordance with ISO 11348-1 (2007) and the Chinese national standards GB/T 21805-2008 and GB/T 18420.2-2009. Standard marine test organisms were used, including the bioluminescent bacterium *Vibrio fischeri*, marine diatom *Chaetoceros muelleri*, cladoceran *Moina mongolica*, anostracan *Artemia salina*, whiteleg shrimp *Litopenaeus vannamei*, and fish *Mugilogobius chulae*. The results showed that sulfonated asphalt and emulsifier 2 were the most toxic of the 26 drilling fluid additives tested, followed by White oil No. 3, anti-high temperature fluid loss additive, cleaning agent, and emulsifier 1. The order of species sensitivity to drilling fluid component toxicity was *M. chulae>M. mongolica>A. salina>L. vannamei>C. muelleri>V. fischeri*. A. salina, *M. mongolica*, and *L. vannamei* and positively correlated with *C. muelleri* and *L. vannamei*. In conclusion, it was determined that *M. chulae* is suitable as a standard laboratory-reared organism for drilling waste toxicity assessments, followed by *M. mongolica* and *C. muelleri*. In view of their comparatively high toxicities, both sulfonated asphalt and emulsifier 2 merit further investigation and should be replaced by more ecologically benign products.

**Keywords:** Toxicity evaluation; Drilling fluid additives; Test organisms; Hierarchical management system

## Introduction

Drilling fluids have undergone a major technological evolution since the first drilling operations were performed in the U.S. They have progressed from a simple mixture of water and clays to complex mixtures of various organic and inorganic constituents. These products improve fluid rheology and filtration. At best, they facilitate drill penetration into heterogeneous geological formations. However, their negative environmental effect steadily increases [1]. Ecotoxicological testing provides information about the possible adverse effects of anthropogenic chemicals on biota. The test data can then be used for the selection of the least hazardous chemicals and the regulation of the chemicals associated with oil production [2]. Globally, extensive toxicity testing has been conducted on drilling fluids and their additives. However, relatively little testing of this kind has been performed in China.

Over the past few decades, several aquatic bioassays have been evaluated to test complex samples. They included a wide variety of model organisms from different trophic levels such as luminescent bacteria [3,4], algae [5-7], cladocerans [8,9], shrimp [10,11], and fish [12]. The responses of individual species to certain chemicals may have important ecological consequences including alterations in community structure. However, the degree to which species differ in terms of their relative sensitivities to various substances is not well established [12].

In the present study, marine species, including the bioluminescent bacterium *Vibrio fischeri*, marine diatom *Chaetoceros muelleri*, cladoceran *Moina mongolica*, anostracan *Artemia salina*, whiteleg shrimp *Litopenaeus vannamei*, and fish *Mugilogobius chulae*, were exposed to a wide range of test concentrations. Twenty-six common drilling fluid additives were evaluated under laboratory conditions to determine their  $LC_{50}$  or  $EC_{50}$ . The aims of the present study were to (a) Assess the risks of offshore drilling fluid additives to related species in the marine environment; (b) Provide a basis for the selection of

environment-friendly drilling fluid additives and (c) Correlate relative sensitivities among species and create a framework for the selection of test species.

# Materials and Methods

#### Sample source and preparation

White oil No.3, base mud (solvent is tap water), base mud (solvent is artificial salt water), sodium soil, and other drilling additives were obtained from China Oilfield Services Limited (Beijing, China).

Simulated seawater is prepared by chemical reagent (chemically pure) and tap water. Each litre of simulated seawater contains 21.86 g NaCl, 3.23 g Na<sub>2</sub>SO<sub>4</sub>, 4.53 g MgCl<sub>2</sub>, 0.93 g CaCl<sub>2</sub>, 0.64 g KCl, 0.17 g NaHCO<sub>3</sub> and 0.02 g Na<sub>2</sub>CO<sub>3</sub>.

Prehydrated bentonite was prepared according to the following procedure. (a) To an appropriate vessel, add 120 g  $\pm$  0.1 g sodium soil and stir at 10,000 rpm whilst adding 1,000 g tap water. (b) After 5 min, stop stirring and scrape the adhesive back into the container. (c) Stir the suspension continuously for >2 h until blended. (d) Let the suspension rest at room temperature for >16 h.

Drilling fluid samples were prepared using simulated seawater,

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prehydrated bentonite, and drilling additives. Formula is shown in Table 1. The following is the sample preparation procedure. (1) Weigh and add prehydrated bentonite and simulated seawater to a 10 L widemouthed container. (2) Stir the suspension at 1,000-3,000 rpm for 20 min. (3) Weigh and slowly add the solute to the suspension. (4) After ~10 min, stop stirring the suspension, scrape the adhesive back into the container, and resume stirring for >2 h until it is fully blended. (5) Allow the mixture to rest at approximate 20°C for >16 h.

## Preparation of the stock solution

Samples and diluted water were mixed and stirred for 20 min at 2,000-3,000 rpm. After letting the mixture rest for 60 min, the supernatant was separated and used as a stock solution. Test solutions were prepared with stock solution and dilution water according to the results of the tests to determine concentration ranges. Water quality parameters (pH, conductivity, and dissolved oxygen) were measured using portable meters (Mettler Toledo pH/Ion meter; Mettler-Toledo, LLC, Columbus, OH, USA; YSI<sup>™</sup> Model 550A Dissolved Oxygen Meter; YSI Inc., Yellow Springs, OH, USA) at the beginning and end of the experiment.

## **Toxicity tests**

Three standard methods were used to determine the toxicity of 26 drilling fluid additives on six test organisms representing different trophic levels. Except for V. fischeri, all species were common and indigenous to China.

Luminescent bacteria acute toxicity: This assay was conducted in accordance with ISO 11348-1 [13]. Freshly prepared bacteria (V. fischeri) were obtained from the First Institute of Oceanography, State Oceanic Administration, China. They were cultivated in 2216E culture medium at 20°C for 30 h on a shaker bed rotating at 180 rpm.

Sample	Artificial saltwater (g)	Prehydrated bentonite (g)	Additive (g)	
Polyacrylamide	7,638	2,807	30	
Block agent 2	7,521	2,807	201	
Emulsifier 1	7,555	2,807	100	
Cleaning agent	7,452	2,807	201	
Xanthan gum	7,638	2,807	30	
Polyalcohol	7,349	2,807	301	
Green lubricants	7,349	2,807	301	
Shale inhibitors	7,521	2,807	201	
Block agent 1	7,521	2,807	201	
sulfonated asphalt	7,521	2,807	201	
Block agent 3	7,521	2,807	201	
Barite	7,486	2,807	708	
Preservatives	7,562	2,807	100	
Clay stabilizer	7,562	2,807	100	
Block agent 4	7,521	2,807	201	
Fluid loss additive 1	7,638	2,807	30	
Modified starch	7,521	2,807	201	
Polyurethane	7,349	2,807	301	
Organic soil	7,579	2,807	201	
Emulsifier 2	7,452	2,807	201	
Fluid loss additive 2	7,521	2,807	201	
Anti-high temperature fluid loss additive	7,521	2,807	201	
Chelating agent	7,590	2,807	100	

Table 1: Drilling fluid preparation.

Fresh bacterial OD600 was >1.6 and the luminous intensity was >4  $\times$  10<sup>6</sup>. These values were measured using a Tecan Spark 10M 96-well microplate reader (Tecan Group Ltd., Mannedörf, Switzerland). The dilution water consisted of 2% (w/v) NaCl. The pH was adjusted to 7.0  $\pm$ 0.2. The procedure was as follows: (a) The diluted water and the samples were taken in a water bath at  $15 \pm 1^{\circ}$ C; (b) Diluted bacterial suspension (20 µL)was added to each well and the luminescence intensities (I0) were measured immediately; (c) Sample (180 µL) at various dilution rates was added to each well in the second, third, fourth, fifth, and sixth microplate rows. The dilution water was added to each well in the first microplate row as blank controls; (d) The test microplate was placed in an incubator at  $15 \pm 1^{\circ}$ C. After 15 min exposure, the Luminescence Intensities (It) were measured; (e) All tests were repeated three times and average luminescence intensities were used in the dose-effect plot.

Algal growth inhibition test: This assay was conducted in accordance with GB/T 21805 [14]. C. muelleri in exponential phase were used in this test, which were cultivated in T2 culture medium at  $25 \pm 2^{\circ}$ C for 2-4 d. The test solutions were prepared by mixing 20 mL stock solution with 20 mL growth medium (f/2 Medium) and inoculum culture. The dilution factor was 5. The initial concentration of algal cells in each test vessel was  $5 \times 10^4$  mL<sup>-1</sup>. All flasks were incubated with agitation under constant light intensity (fluorescent lamp, approximately 3,000 lx) and 24 ± 1°C for 72 h. A control and five treatment groups were prepared. The test design included three replicates for each concentration.

Aquatic animal acute toxicity tests: This assay was conducted in accordance with GB/T 18420.2 [15]. Five equally spaced concentration groups and one control group were used. The concentration ratio was always  $\leq$  2.0. Four replicate vessels were used for each test group, and each vessel contained 10 test organisms. Every 24 h during the exposure assay, M. chulae and L. vannamei were fed small quantities of rotifers and Artemia nauplii, respectively. A. salina and M. mongolica received concentrated chlorella.

## Statistical analysis

The  $LC_{50}$  for the aquatic animals and the  $EC_{50}$  for the luminescent bacteria and C. muelleri were calculated according to GB/T 18420.2 [15], ISO 11348-1 [13], and GB/T 21805 [14], respectively.

A bivariate correlation analysis with a Pearson double-tailed correlation coefficient was performed with SPSS v. 13.0 (IBM Corp., Armonk, NY, USA).

The ranking procedure described by Rojícková-Padrtová and Marsálek [6] was used to compare the relative sensitivities of the test species. The species most sensitive to each drilling fluid component was assigned the lowest number and that which was the least sensitive was assigned the highest number (1 and 6, respectively). If  $EC_{50}/LC_{50}$ exceeded a certain value, the result was excluded from the ranking. The averages of these ranks were calculated for each species.

## Results

## Toxicity test results

The results of the luminescent bacterial toxicity test, the algal growth inhibition test, and the aquatic animal acute toxicity tests (V. fischeri, C. muelleri, M. chulae, M. mongolica, A. salina, and L. vannamei, respectively) are shown in Table 2.

## Sensitivity comparison of test organisms

The mean sensitivity rank of the six test species is presented in

Table 3. The lowest rank was obtained for *M. chulae* which, according to this method, was designated the most sensitive species. It was followed by *M. mongolica* whose mean sensitivity rank was, in fact, very close to that of *M. chulae*. The highest mean sensitivity rank was determined for *V. fischeri*, so it was deemed the least sensitive species. It was followed by *C. muelleri* and *L. vannamei*. *A. salina* was ranked with an average sensitivity.

## **Correlation analysis**

A correlation analysis was conducted based on the toxicity test results of the drilling fluid additives. There was a significant positive correlation between *V. fischeri* and *C. muelleri* (r=0.830; p=0.006). *M. mongolica*, *A. salina*, and *L. vannamei* were significantly and positively correlated with one another (*viz.*, r=0.828, P=0.000 between *M. mongolica* and *A. salina*; r=0.576, p=0.010 between *M. mongolica* and *L. vannamei*; and r=0.597, p=0.007 between *A. salina* and *L. vannamei*). *M. chulae* was significantly positively correlated with *C. muelleri* (r=0.804; p=0.000) and *L. vannamei* (r=0.723; p=0.000).

## Comprehensive toxicity comparison of drilling fluid additives

The different drilling fluid additives were ranked according to their  $EC_{50}$  and  $LC_{50}$  [16] as shown in Table 4. A geometric scale was used as ecotoxicological parameters responses normally follow geometric patterns.  $EC_{50}$  for the luminous bacteria ranged from 0.5%-78%. For the algae,  $EC_{50}$  ranged from 0.5%-32%. In other words, the drilling fluid additives varied from very toxic (lower range limit) to nontoxic (upper range limit). The first interval in the geometric scale was 0.5%-1%. Subsequent intervals increased by a factor of two up to 78% or 32%. The first interval, 0.5%-1%, was assigned 256 points. The points assigned to the subsequent intervals were halved until 78%, which was assigned

one point, or until 32%, which was assigned four points. The LC<sub>50</sub> for the aquatic animals spanned between 0.125% and 32% except for LC<sub>50</sub>  $\leq$  00.125%, which was assigned 256 points. The points assigned to the following LC<sub>50</sub> were halved until 32%, which was assigned zero points.

According to GB/T 18420.1 [17], if the biological toxicity test value is  $\geq$  30,000 mg L<sup>-1</sup> in a Grade One sea area, then the water-based drilling fluid sample conforms to biological toxicity limit requirements. Therefore, when a biological toxicity test value determined for an aquatic animal was  $\geq$  320,000 mg L<sup>-1</sup> (32%), then this sample was considered nontoxic and was assigned zero points. For the luminescent bacterial toxicity and algal growth inhibition tests, the most toxic samples were assigned 256 points. For the luminescent bacterial toxicity test, the lowest obtainable point was four and for the algal toxicity test it was one. The highest combined (total) ranking point of all tests was 1,536, indicating very high toxicity. The total ranking points for all drilling fluid additives tested are compiled in Table 5.

The two most toxic products, sulfonated asphalt and emulsifier 2, had total ranking points of 1,056 and 913, respectively. These were twice as high as the next two substances, White oil No. 3 and antihigh temperature fluid loss additive, whose total ranking points were 549 and 414, respectively. The biotoxicities of cleaning agent and emulsifier 1 were comparatively lower (total ranking points 320 and 297, respectively). Polyurethane, fluid loss additive 2, green lubricants, polyacrylamide, chelating agent, organic soil, and fluid loss additive 1 followed closely behind in terms of biotoxicity. All other products had either relatively low toxicity or were essentially nontoxic. Therefore, they exerted no toxic effects to the luminous bacteria, the algae, or the aquatic animals.

Samala	EC <sub>50</sub> (I	mg·L¹)	LC₅₀ (mg·L⁻¹)				
Sample	V. fischeri	C. muelleri	M. chulae	M. mongolica	A. salina	L. vanname	
Base mud (solvent is tap water)	>1,010,000	>350,000	309,656	>640,000	>640,000	>640,000	
Base mud (solvent is artificial salt water)	>1,000,000	>350,000	132,989	>640,000	>640,000	>640,000	
White oil No. 3	>830,000	>350,000	11,055	8	16	18,477	
Polyacrylamide	>1,010,000	>320,000	46,232	1,939	32,490	>320,000	
Block agent 2	>1,030,000	>320,000	94,044	>320,000	>640,000	>640,000	
Emulsifier 1	>1,020,000	>320,000	10,611	1,015	18,234	76,912	
Cleaning agent	25,600	28,874	4,886	9,548	4,156	7,937	
Kanthan gum	>950,000	>320,000	30,783	51,935	67,608	384,968	
Polyalcohol	>830,000	45,030	13,337	17,679	43,873	116,717	
Green lubricants	227,460	36,467	4,886	5,236	129,960	82,244	
Shale inhibitors	>1,000,000	123,282	23,601	359,188	308,343	274,409	
Block agent 1	432,952	172,306	24,061	20,189	124,012	67,963	
Sulfonated asphalt	120,493	7,730	597	260	684	12,311	
Block agent 3	778,165	>320,000	28,973	102,803	305,549	>640,000	
Barite	>1,060,000	>320,000	>640,000	>640,000	>640,000	>640,000	
Preservatives	686,740	245,527	12,803	91,928	80,009	25,787	
Clay stabilizer	>950,000	397,100	40,394	87,525	114,246	143,103	
Block agent 4	176,542	147,843	27,363	278,576	259,921	530,937	
Fluid loss additive 1	>990,000	>320,000	82,392	2,725	42,404	>640,000	
Aodified starch	425,568	304,229	68,053	64,372	122,735	403,175	
Polyurethane	>780,000	57,135	11,095	3,201	3,969	62,877	
Drganic soil	>850,000	388,240	42,871	4,266	6,373	327,480	
Emulsifier 2	>1,030,000	93,089	1,895	343	733	735	
luid loss additive 2	159,838	17,318	11,149	13,195	80,445	149,988	
Anti-high temperature fluid loss additive	9,894	19,020	12,030	28,918	40,481	156,346	
Chelating agent	>1,000,000	28,695	13,517	21,936	24,061	24,623	

Table 2: Results of toxicity tests.

Test sugarism	Sensitivity score						M
Test organism	1	2	3	4	5	6	Mean sensitivity rank
V. fischeri	1	1	1	1	2	15	5.23
C. muelleri	0	2	4	5	8	2	4.19
L.vannamei	0	1	6	8	5	3	4.13
A. salina	1	7	10	5	2	1	3.12
M. mongolica	13	7	0	3	3	0	2.08
M. chulae	14	8	5	1	1	0	1.86

 Table 3: Frequency of sensitivity score and mean sensitivity rank.

V. fischeri and C. r	nuelleri	Aquatic animal	s
EC <sub>50</sub> (%)	Point	LC <sub>50</sub> (%)	Point
0.5 to ≤ 1	256	≤ 0.125	256
1 to ≤ 2	128	0.125 to ≤ 0.25	128
2 to ≤ 4	64	0.25 to ≤ 0.5	64
4 to ≤ 8	32	0.5 to ≤ 1	32
8 to ≤ 16	16	1 to ≤ 2	16
16 to ≤ 32	8	2 to ≤ 4	8
32 to ≤ 64	4	4 to ≤ 8	4
64 to ≤ 78	2	8 to ≤ 16	2
≤ 78	1	16 to ≤ 32	1
		≤ 32	0

Table 4: Ranking points (Aquatic animals include *M. chulae, M. mongolica, A. salina* and *L. vannamei.*).

# Discussion

According to the luminescent bacterial toxicity tests, the EC<sub>50</sub> of sulfonated asphalt, anti-high temperature fluid loss additive, and cleaning agent were>2,300 mg L<sup>-1</sup> [18], 2412 mg L<sup>-1</sup> [19], and 2,500 mg L<sup>-1</sup> [20], respectively. Thus, they were classified as "slightly toxic". However, the comprehensive biotoxicity test showed that sulfonated asphalt was the most highly toxic and had the highest total score (1,056). In contrast, anti-high temperature fluid loss additive and cleaning agent were assessed as moderately toxic (total score: 414 and 320, respectively).

Emulsifier 2 is used to prepare emulsions of diesel oil, white oil, and synthetic base oil (gas oil) drilling fluids. White oil No. 3 is a base additive for oil drilling fluid. Emulsifier 1 is an important emulsifier and a key additive of oil-based drilling fluid [21]. According to Table 5, emulsifier 2, White oil No. 3, and emulsifier 1 were assigned 913, 540, and 297 points, respectively. Traditionally, Oil-Based Fluids (OBFs) have had poor environmental performance and relatively high ecotoxicity. Base oil was found to be more toxic than drilling mud [22]. The hydrocarbon oils in oil-based mud enter the gills and perturb respiration, the nervous system, blood formation, and enzyme activity [10]. The relatively higher toxicities of sulfonated asphalt, emulsifier 2, White oil No. 3 and emulsifier 1 may be associated with their comparatively higher oil content. For example, the oil content of sulfonated asphalt is 60% [23].

In Norway, the components of the drilling chemicals they use are tested for toxicity on algae, shrimp, and juvenile fish. They require operators on the Norwegian Continental Shelf to classify offshore chemicals as "green", "yellow", "red" or "black". Norway ordinarily

Sample Point									
Sample	V. fischeri	C. muelleri	M. chulae	M. mongolica	A. salina	L. vannamei	Total		
Sulfonated asphalt	16	256	256	256	256	16	1,056		
Emulsifier 2	1	16	128	256	256	256	913		
White oil No. 3	1	4	16	256	256	16	549		
Anti-high temperature fluid loss additive	256	128	16	8	4	2	414		
Cleaning agent	64	64	64	32	64	32	320		
Emulsifier 1	1	4	16	256	16	4	297		
Polyurethane	1	32	16	64	64	4	181		
Fluid loss additive 2	16	128	16	16	2	2	180		
Green lubricants	8	64	64	32	2	2	172		
Polyacrylamide	1	4	4	128	8	0	145		
Chelating agent	1	64	16	8	8	8	105		
Organic soil	1	4	4	64	32	0	105		
Fluid loss additive 1	1	4	2	64	4	0	75		
Polyalcohol	1	32	16	16	4	2	71		
Preservatives	2	8	16	2	2	8	38		
Block agent 1	4	8	8	8	2	4	34		
Block agent 4	8	16	8	1	1	0	34		
Shale inhibitors	1	16	8	0	1	1	27		
Modified starch	4	8	4	4	2	0	22		
Xanthan gum	1	4	8	4	4	0	21		
Block agent 3	2	4	8	2	1	0	17		
Clay stabilizer	1	4	4	2	2	2	15		
Block agent 2	1	4	2	0	0	0	7		
Base mud (solvent is artificial salt water)	1	4	2	0	0	0	7		
Base mud (solvent is tap water)	1	4	1	0	0	0	6		
Barite	1	4	0	0	0	0	5		

Table 5: Total points for the offshore drilling chemicals based on the summary of the EC<sub>50</sub> and LC<sub>50</sub> values.

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permits zero discharge of all offshore chemicals rated "red" or "black". However, "red" chemicals may be discharged if an operator can prove that no other options were available, and an active search is underway to replace or substitute that product [24]. According to the Norway waste management hierarchy, sulfonated asphalt and emulsifier 2 should be classified as "red" or "black", prioritized for substitution, and strictly regulated. White oil No. 3, anti-high temperature fluid loss additive, cleaning agent, emulsifier 1, polyurethane, fluid loss additive 2, green lubricants, polyacrylamide, chelating agent, and organic soil were rated practically nontoxic or nontoxic. Therefore, they should be designated "yellow" and would usually not be defined as hazardous. Fluid loss additive 1, polyalcohol, preservatives, block agent 1, block agent 4, shale inhibitors, modified starch, xanthan gum, block agent 3, clay stabilizer, block agent 2, base mud (solvent is artificial salt water), base mud (solvent is tap water), and barite should be classified as "green" and are not expected to damage or be harmful to the marine environment.

In general, the European approach is directed towards the control of chemical use (potential chemical environmental toxicity) whereas the American approach is directed towards the control of final emissions (actual effluent environmental toxicity) [2]. In the past, China conducted ecotoxicological testing and based their drilling waste regulations on the American model. Evidently, our knowledge regarding the overall effect of drilling fluid additives on marine ecosystems is limited. The multispecies toxicity testing used in the present study provided a basis for the selection of environmentally friendly drilling fluid additives. If the hierarchical management system were adopted, chemicals classified as "red" or "black" would be prioritized for substitution and strictly regulated whereas the application of those rated "green" would be promoted. In this way, the discharge of toxic drilling fluid additives could be reduced. Regarding sulfonated asphalt, a form of sulfonated asphalt has been developed which has similar properties to sulfonated asphalt itself but without the toxicity [25].

The marine luminescent bacterium *Vibrio fischeri* is widely used in toxicity testing and easily, quickly, and reliably generates toxicity data for chemicals and wastewater [4]. In the present study, however, *V. fischeri* ranked as the least sensitive of all six species tested. Moreover, the correlations of the toxicity data between *V. fischeri* and the other aquatic species were poor. Furthermore, the relative growth of luminescent bacteria is significantly influenced by pH, chromaticity, turbidity, and residual chlorine [26]. In addition, as prokaryotes, luminescent bacteria do not fully represent the acute toxicities of pollutants to all living organisms [27]. *Vibrio fischeri* may be useful in preliminary toxicity testing but it is not ideal for the evaluation of drilling waste toxicity.

Microalgae are the primary producers and are situated at the base of the aquatic food chain. They are among the first to be affected by environmental contamination. Consequently, they provide important information for predicting the effect of pollution in aquatic ecosystems [7]. In this study, *C. muelleri* was slightly less sensitive to the drilling fluid additives than the other taxa. Nevertheless, its test data correlated well with those for *A. salina* and *L. vannamei* and it is still recommended as a test species for drilling waste toxicity assessments.

*M. mongolica* is distributed through North Africa and across the Middle East, Central Russia, and Mongolia. It tolerates a wide range of salinities, reproduces rapidly, grows easily in culture, and is a good live food source for marine fish larvae [8]. White leg shrimp (*L. vannamei*) is suitable for ecotoxicological testing because it is commercially and ecologically important and it is highly sensitive to various anthropogenic chemicals [11]. When using Artemia in toxicological testing, there are several practical considerations including cyst production, ecological

relevance, systematic use, and the maintenance and sustainability of laboratory culture conditions [28]. In the current study, *M. mongolica*, *A. salina*, and *L. vannamei* had very similar average sensitivities, were closely correlated, and were ranked equally. As it is of a convenient size and is comparatively easy to handle and artificial breeding, we recommend *M. mongolica* as a standard marine test organism for water pollution assessments.

The yellow stripe goby (*M. chulae*; family Gobiidae) is a small egglaying marine teleost distributed along the coastal areas of the Western Pacific region including China, Japan, the Philippines, Indonesia, Vietnam, and Thailand [29]. Previous studies have indicated that *M. chulae* has excellent potential as a laboratory fish because of its small body size, short reproductive cycle, high fecundity, ease of culturing, and sensitivity to pollutants [30]. In this study, it was determined that *M. chulae* was the most sensitive of all six species tested. Moreover, the toxicity test results of *M. chulae* were significantly correlated with those for *C. muelleri* and *L. vannamei*. For these reasons, we recommend *M. chulaeas* a fish model for drilling waste toxicity testing.

No single biological response or test species can meet all the environmental and legislative requirements for effective toxicity testing [31]. Organism sensitivity varies substantially with the type of pollutant. The concept of the "most sensitive species" is a myth [32]. For example, *M. chulae* is >20 × more sensitive to sulfonated asphalt and 16 × more sensitive to green lubricants than *L. vannamei*. On the contrary, *L. vannamei* is significantly more sensitive to emulsifier 2 than *M. chulae*.

## Conclusion

In the toxicity test of 26 drilling fluid additives, *M. chulae* showed better sensitivity and correlation compared with other organisms. Along with meeting the basic requirements as a model organism for the study of aquatic ecological toxicology, *M. chulae* is an ideal test organism with good application prospects for testing the toxicity of drilling fluid waste. Furthermore, *M. mongolica* and *C. muelleri* can also be used as candidate organisms considering the representativeness of test species with different trophic levels and the possibility of artificial breeding. In view of their comparatively high toxicities, both sulfonated asphalt and emulsifier 2 merit further investigation and should be replaced by more ecologically benign products.

Drilling fluid components are complex. Therefore, a scientific approach to biotoxicity assessment and grading will help improve the environmental performance of drilling waste. Furthermore, it is necessary to use a wide variety of assays to obtain complete aquatic toxicity assessments. Test species representing different trophic levels should be selected.

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