

Acute toxicity of aqueous film forming foam (AFFF) to zebrafish (*Brachydanio rerio*)

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Abstract. The usage of aqueous film forming foam (AFFF) and other firefighting foams improves the effectiveness of firefighting operations and is considered as a significant factor for carrying out a successful firefighting action. As public awareness has increased, concerns have been raised about the impact AFFFs have on the environment in recent years. In this paper, the acute toxicity of AFFF to zebrafish was conducted to classify the acute toxicity level of 14 AFFF products from 7 different manufacturers on domestic market. The test results showed that the 96h LC₅₀ values of 14 kinds of AFFF ranged from 232.57 to 5501.04 mg/L, which was comparable to foreign AFFF products. The products from different sources own different level of toxicity. For the AFFF products of the same formula system, the addition of alcohol-resistant components enhanced the acute toxicity of AFFF, while the addition of antifreezing components reduced the toxicity of AFFF.

1. Introduction

Because of its low surface tension, aqueous film forming foam (AFFF) is the most effective foam to extinguish Class B fires (flammable, volatile liquids) worldwide. Each year, plenty of AFFF is used to extinguish Class B fires and released into the environment after fire. As public awareness and environmental regulations have increased, concerns have been raised about the impact AFFF and its ingredients have on the environment.

AFFF and other firefighting foams have several intrinsic properties that may cause environmental impacts, including aquatic toxicity, biodegradability, oxygen demand and foaming. Toxicity analysis focuses on the plants or animals most likely affected by firefighting foams, and the route of entry that will cause the worst effects. Aquatic toxicity is an indicator of the relative toxicity of a chemical or compound in water. So it is the most important property when considering AFFF's environmental impacts. Aquatic toxicity is determined by using a series of tests for acute (short term), expressed as EC₅₀ (Effective Concentration 50), LC₅₀ (Lethal Concentration 50), or other several other parameters.

The zebrafish (*Brachydanio rerio*) is a freshwater fish belonging to the minnow family (Cyprinidae) of the order Cypriniformes. The zebrafish is an important and widely used vertebrate model organism in scientific research. It is particularly notable for its regenerative abilities, and has been modified by researchers to produce many transgenic strains.

The aim of the research presented in this paper was to evaluate and compare the acute toxicity of AFFF samples commonly used by China fire services using zebrafish (*Brachydanio rerio*). Also the acute toxicity of different types of AFFF products were analyzed to definite the rules of different ingredients played in the acute toxicity.



2. Materials and methods

2.1 AFFF samples

In order to achieve suitable foaming and functional properties, AFFF and other firefighting foams usually contain many additional substances, such as fluorinated surfactants, carbon hydrogen surfactants, organic solvents and other ingredients.

According to GB 15308-2006 “Foam extinguishing agent” in China, AFFF can be classified as common AFFF and AFFF/AR, which is alcohol-resistant AFFF. Also AFFF concentrate with different freezing points are developed to be suitable for use at different temperatures.

The AFFF formulas from different manufacturers differ from one another. But the AFFF products from one manufacturer may use the same formulation system. So to evaluate the effect of mixing ratio, the alcohol-resistant and antifreeze ingredients on the toxicity of AFFF, 14 AFFF samples from 7 different manufacturers in China were collected.

AFFF and other firefighting foam usually come in a concentrate that is diluted with water and agitated to form a foam solution. The details of 14 AFFF concentrate samples are shown in Table 1.

Table 1. Specifications of AFFF samples

No.	Manufacturer	AFFF types
1#	A	6%(AFFF, -13 °C)- Sea water resistance
2#		6%(AFFF/AR, -12 °C)- Sea water resistance
3#	B	3%(AFFF, -11 °C)- Sea water resistance
4#		3%(AFFF, -30 °C)- Sea water resistance
5#		3%(AFFF/AR, -30 °C)- Sea water resistance
6#	C	3%(AFFF, -6 °C)
7#		3%(AFFF, -25 °C)- Sea water resistance
8#		6%(AFFF, -10 °C)- Sea water resistance
9#		3%(AFFF/AR, -6 °C)
10#		6%(AFFF/AR, -5 °C)
11#	D	6% (AFFF, -10 °C) - Sea water resistance
12#	E	6% (AFFF, -10 °C) - Sea water resistance
13#	F	3% (AFFF, -30 °C) - Sea water resistance
14#	G	3% (AFFF, -25 °C)

2.2 Fish species

Zebrafish (*Brachydanio rerio*) was selected as test fish in this study. In China, Zebrafish are easy to rear and widely available throughout the year, and they are commonly used in ecotoxicity test. The average body length of fish was 3.0 ± 0.5 cm, and the body weight was 0.3 ± 0.1 g. The fishes were obtained and held in the laboratory for at least 12 days before used for testing. The fishes were feeded daily until 24 hours before the test was started.

2.3 Test procedure

In this study, the static test method was employed to evaluate the toxicity of AFFF concentrate according to OECD 203: Fish, Acute Toxicity Test. Series volumes of AFFF concentrate were added into several 2L glass beakers, and standard dilution water was added until the volume was 2 L. The test solutions were stirred evenly using glass rod, and the foam formed on the surface of solution was removed from the surface. At least 5 concentrations in a geometric series were set to get the accurate

results. At the same time, potassium dichromate solution of different concentrations were used as toxicity reference. The blank control team containing standard dilution water only was run in addition to the test series. The temperature of the test solution was constant on 23 ± 1 °C.

In each test beaker, 10 fish were added to start the test, and the fish are exposed to the test substance preferably for a period of 96 hours. Each day, the photoperiod was 12 to 16 hours, and no feeding during the test period. Mortalities are recorded at 24, 48, 72 and 96 hours and the concentrations which kill 50 per cent of the fish (LC_{50}) are determined where possible. The fish were inspected daily during the test period. Fish were considered dead if there was no visible movement (e.g. gill movements) and if touching of the caudal peduncle produces no reaction. Dead fish were removed from the beakers when observed and mortalities were recorded. The fish dead rate of blank control team should be less than 10% during 96 hours, and the LC_{50} value of toxicity reference group was between 200 and 400 mg/L.

The cumulative percentage mortality for 96 hours was plotted against concentration on logarithmic probability paper. Normal statistical procedures were employed to calculate the LC_{50} of 96 hours.

3. Results and discussion

3.1. Acute toxicity level of AFFF in China

The experimental photo of fish acute toxicity test of AFFF and toxicity reference is shown in Fig. 1. In experimental photo, the yellow beaker is potassium dichromate solution as toxicity reference, and the left 3 beakers are AFFF solution of different concentration. The LC_{50} values of 14 AFFF samples commonly in China is shown in Table 2.

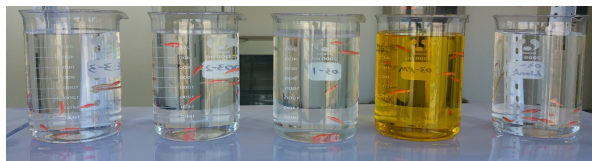


Fig. 1 Experimental photo of fish acute toxicity of AFFF

Table 2 The LC_{50} of 11 AFFF samples in China

No.	Mixing ratio (%)	Sample type	Freezing point (°C)	LC_{50} value (mg/L)	<i>P</i> -value
1#	6	AFFF	-13	2571.07	0.9842
2#	6	AFFF/AR	-12	1441.52	0.9931
3#	3	AFFF	-11	812.41	0.9904
4#	3	AFFF	-30	5501.04	0.9254
5#	3	AFFF/AR	-30	2212.53	0.8763
6#	3	AFFF	-6	301.05	0.8607
7#	3	AFFF	-25	2750.43	0.9633
8#	6	AFFF	-10	1502.36	0.9142
9#	3	AFFF/AR	-6	232.57	0.9654
10#	6	AFFF/AR	-5	275.71	0.8935
11#	6	AFFF	-10	1888.89	0.9273
12#	6	AFFF	-10	1250.12	0.9801
13#	3	AFFF	-30	1245.55	0.9432
14#	3	AFFF	-25	364.45	0.8955

LC_{50} is one of commonly used parameters in ecotoxicity study to evaluate the acute toxicity. This parameter is counterintuitive, so the higher the number, the less toxic the material. From Table 2 we can see that the 96h LC_{50} values of 14 AFFF samples in China are from 232.57 to 5501.04 mg/L, showing the range of acute toxicity of AFFF in China is wide. Among all the listed samples in this paper, the LC_{50} values of most samples lie between 800 and 3000 mg/L, while 4 samples are below 500 mg/L and 1 sample beyond 5000 mg/L. The data mentioned above shows that the AFFF samples from different manufacturers have different levels toxicity to fish.

The AFFF concentrate used in China is usually divided into 3% and 6% type, according to the mixing ratio when it is diluted with water. From the acute toxicity data given in Table 3, the LC₅₀ of 3% AFFF samples are much lower than that of 6% samples, indicating the acute toxicity of 3% AFFF concentrate is much higher than 6% AFFF. In practise, 3% AFFF concentrate is high concentrated compared to 6% concentrate, so the content of ingredients in 3% concentrate is higher than 6% concentrate. This leads to the toxicity difference between 3% and 6%.

The acute toxicity data discussed above are focusing on AFFF concentrate. The 96h LC₅₀ data indicates that AFFF concentrate is toxic. Actually, when it is used to extinguish fire, AFFF concentrate is diluted with plenty of water to form AFFF solution. AFFF is also released into the environment in the form of AFFF solution. So after diluted, AFFF solution is typically categorized as relatively harmless. On this point, the risk of aquatic toxicity of AFFF solution to fish is very low, compared to AFFF concentrate and other chemicals, such as commercial catering detergents and so on.

3.2 Fish acute toxicity comparison of AFFF at home and abroad

In China, the environmental impact of AFFF and other firefighting foam attracts public attention only in recent years. So the report of fish acute toxicity of AFFF in China is relatively few compared to European and American countries, also the toxicity data of AFFF in China is rare. Therefore the current toxicity data reported is mainly from abroad. The summary of 96h LC₅₀ values of AFFF samples reported abroad is shown in Table 3.

Table 3 96h LC₅₀ values of AFFF abroad

AFFF samples	Fish species	96h LC ₅₀ (mg/L)
Milspec AFFF	Fingerling Rainbow Trout	2176
AFFF/AR	Fingerling Rainbow Trout	3536
UL AFFF	Fingerling Rainbow Trout	5657
Chemguard 3%AFFF	Pimephales promelas	233

The 96h LC₅₀ values of AFFF abroad shown in Table 4 ranges from 233 to 5657 mg/L, which means the AFFF samples from different manufacturers abroad have big difference in acute toxicity. This result may due to the difference in formulation system manufacturer employed.

The 96h LC₅₀ values of 14 AFFF samples in China are from 232.57 to 5501.04 mg/L, so the 96h LC₅₀ values of AFFF samples in China and abroad are in the same range, meaning that the fish acute toxicity of AFFF samples in China and abroad are at the same level. In aquatic ecotoxicity test, the usage of different fish species may lead to big difference of the acute toxicity data. But the fish species employed in this study and abroad are all classic model biology, their toxicity data has been verified by lots of experiments. So the toxicity data in Table 2 and 4 is comparable. The LC₅₀ values in this paper and abroad is at the same range although using different fish species. The results indicate that the fish acute toxicity of AFFF samples in China and abroad are at the same level.

In most firefighting foam product standards in China and abroad, there is rare requirement on acute toxicity of AFFF products. Only in MIL-F-24385F "Fire Extinguishing Agent, Aqueous Film-forming Foam (AFFF) Liquid Concentrate, for Fresh and Sea Water", the minimum 96h LC₅₀ value should be 500mg/L for 3% AFFF, and 1000mg/L for 6% AFFF concentrate respectively while the test fish is Killiefish (*Fundulus heteroclitus*). Without considering the fish species employed, the 96h LC₅₀ data of most AFFF samples in this paper is in accordance with the requirement of MIL-F-24385F.

3.3 Acute toxicity difference of AFFF and AFFF/AR

The alcohol-resistant AFFF (AFFF/AR) is specially used to extinguish water soluble liquid fire. In this study, the acute toxicity difference of typical AFFF and AFFF/AR is evaluated, and the LC₅₀ of AFFF and AFFF/AR is shown in Fig. 2.

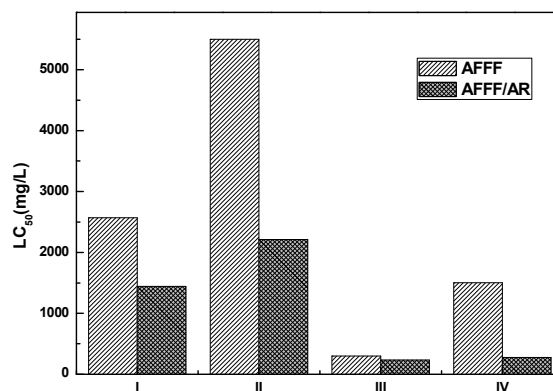


Fig. 2 LC₅₀ of typical AFFF and AFFF/AR

As discussed above, the AFFF samples with different formulation system have great difference in acute toxicity. So when considering the acute toxicity difference between AFFF and AFFF/AR, the foam samples should come from the same formulation system. In this part, four groups of AFFF and AFFF/AR are from four factories. In each group of AFFF and AFFF/AR samples, the 96h LC₅₀ values of AFFF are all higher than AFFF/AR respectively, especially for the samples from group I, II and IV. The results mean that for the same formulation system, the acute toxicity of AFFF/AR is much higher than AFFF without alcohol-resistant.

Generally, xanthan gum and other macromolecule polysaccharides are usually added in AFFF as alcohol resistant ingredient. When the alcohol-resistant AFFF foam is applied on the surface of water soluble liquid, the polysaccharide in the foam will separate out from foam quickly and form a stable glue membrane on the surface of water soluble liquid to resist the destruction of water soluble liquid to foam bubbles.

Generally, xanthan gum and other polysaccharides are natural products extracted from organisms, so they have low toxicity, which have been proved by other studies. At the same time, as natural products, these ingredients are also easily to be biodegraded by the microorganism in water and air. To prevent the deterioration and prolong the shelf life, bactericide, such as formaldehyde and phenol are usually added to AFFF/AR. According to previous research data, the 96h LC₅₀ values of formaldehyde to *Ictalurus punctatus* and *Lepomis cyanellus* are 24.8 and 69.2 mg/L, indicating it is extremely toxic. So the addition of bactericide in AFFF/AR increases its acute toxicity, and leads to the higher toxicity of AFFF/AR than AFFF.

3.4 Acute toxicity difference of AFFF samples with different freezing points

China has a vast territory and there is a great temperature difference between the north and the south. So AFFF products with different freezing points are necessary to insure its usage at different latitudes.

Among the 14 AFFF samples collected in this study, 3# and 4#, 6# and 7# are the AFFF products from plant B and C respectively. They have the same basic formulation each other, while the different freezing points are different. The data in Table 2 shows that the LC₅₀ of 3# (-11°C) and 4# (-30°C) are 812.41 and 5501.04 mg/L, while the LC₅₀ of 6# (-6°C) and 7# (-25°C) are 301.05 and 2750.43 mg/L respectively. The results show that the acute toxicity decreases with the decrease of the freezing point. The results of other firefighting foam we tested also show the same trend (data not mentioned here).

Short chain alcohols are usually chosen as anti-freeze agent in firefighting foam formula. In China, the frequently-used short chain alcohols are ethylene glycol and glycerol. The ecotoxicology data shows that the 96h LC₅₀ of ethylene glycol to blue gill sunfish is 100000 mg/L, while the 96h LC₅₀ of glycerol to rainbow trout is 184000 mg/L. According to the criteria of acute toxic grade of chemical, ethylene glycol and glycerol are relatively harmless. The more the anti-freeze agent added to AFFF formula, the lower the freezing point of AFFF concentrate. The addition of plenty of low toxic anti-freeze agent leads to the acute toxicity of AFFF product as a whole decreased.

4. Conclusions

In this study, 14 AFFF samples of different specifications commonly used by China fire service were tested to evaluate and compare the acute toxicity of AFFF samples in China. Also the acute toxicity of different types of AFFF products were analyzed to definite the rules of different ingredients played in the acute toxicity. The results are as follows:

(1) The 96h LC₅₀ of 14 AFFF concentrate samples from 7 manufacturers range between 232.57 and 5501.04 mg/L, most samples are low toxic. There is a great difference in the acute toxicity of AFFF asmples from different manufacturers. The acute toxicity of 3% AFFF concentrates are commonly higher than 6% AFFF concentrates.

(2) The 96h LC₅₀ of AFFF samples in China and abroad are in the same range, meaning the fish acute toxicity of AFFF samples in China and abroad are at the same level. The 96h LC₅₀ data of most AFFF samples in this paper is in accordance with the requirement of MIL-F-24385F.

(3) For the AFFF and AFFF/AR samples of the same formulation system, the acute toxicity of AFFF/AR is much higher than AFFF without alcohol-resistant. This is mainly due to the addition of highly toxic formaldehyde and other preservative to AFFF/AR.

(4) The acute toxicity decreases with the decrease of the freezing point. The reason is that the addition of plenty of low toxic anti-freeze agent leads to the acute toxicity of AFFF product as a whole decreased.

Acknowledgements

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