

Novel Juice
Fish, Acute Toxicity Test :
STUDY REPORT

Client: TAQKEY Science
Testing Institution: SGS TAIWAN LTD.
Report No.: UB/2014/40329A-01
Report Date: 2014.06.03

- Note:**
1. The report is separated for used in vain.
 2. N.D. Non-detected means the test results are lower than detection limit value.
 3. Any unauthorized alteration, forgery or falsification of the content or appearance of this report is unlawful and offenders may be prosecuted to the fullest extent of the law.

STUDY SCHEDULE
Fish, Acute Toxicity Test :
Novel Juice

Report No.:	UB/2014/40329A-01
Test article registration date:	2014.04.28
Experimental starting date:	2014.05.18
Experimental completion date :	2014.05.23
Study completion date:	See study Director's signature date in the report



Testing Institution

Name: SGS TAIWAN LTD.

Address: No. 38, Wu Chyuan 7th Rd., New Taipei Industrial Park, Wu Ku Dist., New Taipei City 24890, Taiwan (R.O.C)

Client / Sponsor

Name: TAQKEY Science

Address: 1F., NO. 60, Jiabei 2nd St., Zhunan Township, Miaoli County 350

INFORMATION FOR TEST ARTICLE SHEET

04/14/2014/周: 05:22 PM

FAX No.

P. 002



INFORMATION FOR TEST ARTICLE / CONTROL ARTICLE

Sponsor Company Name		TAQKEY Science	
Sponsor Address		1F., No.60, Jiabei 2nd St., Zhunan Township, Miaoli County 350	
Contract study item		<input checked="" type="checkbox"/> Base on the contract <input type="checkbox"/> Others	
Name of Test article/ Control article	Novel Juice		
Batch/Lot number	<input checked="" type="checkbox"/> Base on the specific number on the package : <u>LD11327294</u>		
	<input type="checkbox"/> Base on the date on the package : _____		
	<input type="checkbox"/> Base on the arrived date		
	<input type="checkbox"/> Others : _____		
Specification & Amount	1000ul / vial * 9 vial (e.g.10ml / bottle * 6 bottles)		
Retention amount (Note 2)	The amount of the same lot is sufficient for <input type="checkbox"/> One test <input checked="" type="checkbox"/> Two test (for retention)		
External features	External features: <input checked="" type="checkbox"/> liquid <input type="checkbox"/> powder <input type="checkbox"/> tablet <input type="checkbox"/> capsule <input type="checkbox"/> Other		Color : <u>Green</u>
Major components & Purity	Major components: <u>glycerol</u>		Purity: <u>up to 90%</u>
Solvent and solubility	N/A		
Storage condition	<input type="checkbox"/> Room temperature <input checked="" type="checkbox"/> 4°C <input type="checkbox"/> Dry <input checked="" type="checkbox"/> Light sensitive <input type="checkbox"/> Others		
Expiration date (Note 3)	<input checked="" type="checkbox"/> Date: <u>2016/02/01</u> (YYYY/MM/DD) or <input type="checkbox"/> Period : _____ year _____ month _____ day		
Attachment (Note 4)	<input type="checkbox"/> Certificate of Analysis <input checked="" type="checkbox"/> Material Safety Data Sheet <input type="checkbox"/> Stability Test Result <input checked="" type="checkbox"/> Other : <u>Protocol</u> <input type="checkbox"/> No attachment (Note4)		
Sterilization	Has been sterilized <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO (If Yes, please select the following item) Methods <input type="checkbox"/> EO sterilization <input type="checkbox"/> Gamma sterilization <input type="checkbox"/> Steam sterilization <input type="checkbox"/> Other		
Categorization of devices (The column is only for device used)	1. <input type="checkbox"/> Contact with intact skin or mucosa (cumulative contact duration) <input type="checkbox"/> Short-term (no greater than 4 hr) <input type="checkbox"/> Long-term (exceeding 4 hr) Maximum duration is _____ hrs 2. <input type="checkbox"/> Implanted device		
Specific requirement (Note 5)	<u>Dilute Ratio : 500mg/L. (Fish Toxicity).</u>		
Sponsor Signature/Date : <u>[Signature]</u> <u>04 Apr. 2014</u>			
<p>Note 1. Above all information is disclosure by the sponsor.</p> <p>Note 2. If the sponsor doesn't provide the retention of test article/control article, the retention of a reserved test article/control article from each batch of test article /control article is the responsibility of the Sponsor.</p> <p>Note 3. If the effective period is less than 5 years, the test article/control article will be retained till the expiry date. If the effective period is longer than 5 years, the test article/control article will be retained for 5 years only.</p> <p>Note 4. Determination and documentation of identity, strength, purity, stability, composition, method of synthesis, fabrication, derivation or other characteristics of the test article/control article are the responsibility of the Sponsor.</p> <p>Note 5. The test article/control article which has been destroyed or cutting will be discarded after the end of experiment. For retention or return of the kind of test article/control article, please indicate in the "special requirement". The human intake suggests or dose requested by the sponsor also can fill in the "special requirement". Note treatment method after test if the test article need to be retreated</p> <p>Note 6. The code number of test article is the same as the report number.</p> <p>Note 7. Note 'N/A' if not applicable. Do not leave blank.</p>			

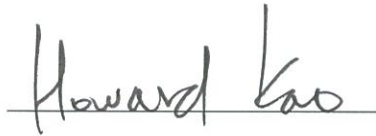
版次 : 3.1 試驗-對照物質資料表 Information for test article-control article
發行日期 : 2013.06.14

APPROVAL SIGNATURE PAGE

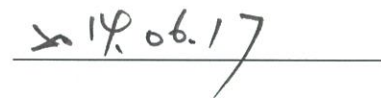
Fish, Acute Toxicity Test :

Novel Juice

Study Director:

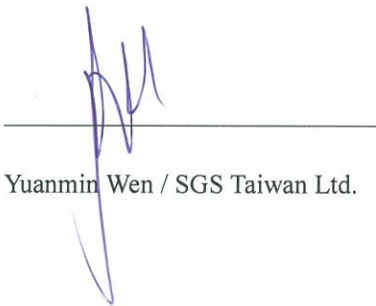


Howard Kao / SGS Taiwan Ltd.

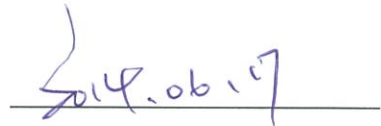


Date Completed

Facility Manager:



Yuanmin Wen / SGS Taiwan Ltd.



Date Completed



TABLE OF CONTENTS

ABSTRACT	7
INTRODUCTION	8
MATERIALS AND METHODS	9
DATA MANAGEMENT	12
RESULTS	13
CONCLUSION	15
REFERENCES	16

ABSTRACT

The study was to evaluate the test article, “Novel Juice” (supplied by TAQKEY Science) for its acute aquatic toxicity. The fish were exposed to the test substance preferably for a period of 96 hours. Mortalities were recorded at 24, 48, 72 and 96 hours, and the concentrations, which would kill 50 per cent of the fish (LC50), were determined where possible.

In the study, the mortality in the controls was 0 per cent at the end of the test. Furthermore, there was not any mortality in the group of fish that exposed to the 0.5 ml/L. The result of the study showed that the LC50 of the acute aquatic toxicity was >0.5 ml/L of “Novel Juice”.

INTRODUCTION

This study was to assess the acute toxicity of the test substance. Mortalities of *Cyprinus carpio* were recorded at 24, 48, 72 and 96 hours, and the concentrations, which killed 50 per cent of the fish (LC50), were determined where possible. The experiment was performed in compliance with the OECD 203.

MATERIALS AND METHODS

A. *Cyprinus carpio* :

Total length of each tested fish was 2-4 cm. They can be bred and cultivated either in fish farms or in the laboratory, under disease- and parasite-controlled conditions, so that the test fish will be healthy and of known parentage.

B. Reconstituted water (for test solution) :

Per liter of the reconstituted water, it contains: 96.0 mg NaHCO_3 , 123.0 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 4.0 mg KCl, and 60.0 mg $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$.

The reconstituted water has been strongly exposed to air until 48 hours before the test day.

C. Holding of fish :

All fish must be obtained and held in the laboratory for at least 12 days before they are used for testing. They must be held in water of the quality to be used in the test for at least seven days immediately before testing and under the following conditions:

Light: 12-16 hours photoperiod daily;

Temperature: $22 \pm 2^\circ\text{C}$;

Oxygen concentration: at least 80 per cent of air saturation value;

Feeding: daily until 24 hours before the test is started.

Following a 48-hour settling-in period, mortalities are recorded and the following criteria are applied:

Mortalities of greater than 10 percent of the population in seven days: rejection of the entire batch; Mortalities of between 5 and 10 percent of the population: acclimatization continues for seven additional days; Mortalities of less than 5 percent of the population: acceptance of the batch.

D. Water :

Good quality natural water or reconstituted water is preferred. Waters with a total hardness of between 10 and 250 mg CaCO₃ per liter and with a pH 6.0 to 8.5 are preferable. The reagents used for the preparation of reconstituted water should be of analytical grade, and the deionised or distilled water should be of conductivity equal to or less than 10 µScm⁻¹.

E. Test solutions :

Test solutions of chosen concentrations are prepared by dilution of a stock solution. The test should be carried out without adjustment of pH. If there is evidence of marked change in the pH of the tank water after addition of the test substance, it is advisable that the test be repeated and adjusting the pH of the stock solution to that of the tank water before addition of the test substance. This pH adjustment should be made in such a way that the stock solution concentration is not changed to any significant extent and that no chemical reaction or precipitation of the test substance is caused. HCl and NaOH are preferred.

F. Procedure :

1. Conditions of exposure.

Duration: 96 hours

Loading: maximum loading of 1.0 g fish/liter

Light: 16 hours photoperiod daily

Temperature: $22 \pm 2^{\circ}\text{C}$

Oxygen concentration: not less than 60 per cent of the air saturation value

Feeding: none

Disturbance: disturbances that may change the behavior of the fish should be avoided

2. Number of fish

20 fish were used at each test concentration and in the controls. Each container carried 10 fish and 1.5L test solution.

3. Test concentrations

5 concentrations were performed and all concentration groups were duplicated. A range-finding test conducted before the definitive test enabled the choice of the appropriate concentration range.

4. Controls

One blank (20 fish): Each container carried 10 fish and 1.5L reconstituted water

5. Observations

The fish were inspected at least after 24, 48, 72 and 96 hours. Fish were considered dead if there was no visible movement (e.g. gill movements) and if touching of the caudal peduncle produced no reaction. Dead fish were removed when observed and mortalities were recorded. Observations at three and six hours after the start of the test are desirable. Records were made on visible abnormalities (e.g. loss of equilibrium, swimming behavior, respiratory function, pigmentation, etc.). Measurement of pH, dissolved oxygen and temperature should be carried out at least daily.

DATA MANAGEMENT

For statistical analysis, data were analyzed using one of the software: Graphic method, Probit method, Sperman-karber method, and Trimmed spearman-karber method. The result showed “LC50 > the highest concentration”, if the mortalities of 5 sample concentrations were less than 50%.

RESULTS

A. Water quality characteristics (pH, oxygen concentration, and temperature)

Table 1.1. pH of test solutions

		Concentration (mL/L)		0 (Control)	0.03	0.06	0.12	0.25	0.50
		Test solution							
pH	0 hr			8.80	7.82	7.85	7.81	7.78	7.53
	24 hr			7.93	7.42	7.36	7.33	7.32	7.30
	48 hr			7.82	7.34	7.26	7.27	7.24	7.26
	72 hr			7.53	7.22	7.25	7.22	7.24	7.24
	96 hr			7.33	7.18	7.10	7.20	7.15	7.08

Table 1.2. Oxygen concentration of test solutions

		Concentration (mL/L)		0 (Control)	0.03	0.06	0.12	0.25	0.50
		Test solution							
Oxygen concentration (mg/L)	0 hr			7.99	7.47	7.51	7.47	7.59	7.50
	24 hr			4.08	3.87	3.43	3.82	3.78	3.85
	48 hr			3.92	3.61	2.05	3.01	2.58	3.64
	72 hr			3.88	3.27	1.87	2.37	2.05	2.89
	96 hr			3.79	3.16	1.55	2.05	1.88	2.15

Table 1.3. Temperature of test solutions

		Concentration (mL/L)		0 (Control)	0.03	0.06	0.12	0.25	0.50
		Test solution							
Temperatrue (°C)	0 hr			22.9	22.9	22.9	22.9	22.9	22.9
	24 hr			22.6	22.6	22.6	22.6	22.6	22.6
	48 hr			22.7	22.7	22.7	22.7	22.7	22.7
	72 hr			22.5	22.5	22.5	22.5	22.5	22.5
	96 hr			22.6	22.6	22.6	22.6	22.6	22.6

B. A range-finding test and definitive test

Table 2.1. Range-finding test

Concentration(mL/L)	Mortality	Number of fish	Ratio of mortality (%)
0(Control)	0	5	0
0.02	0	5	0
0.1	0	5	0

Table 2.2. Definitive test

Concentration (mL/L)	Mortality						Mortality (total)	Ratio (%)
	2 hr	6 hr	24 hr	48 hr	72 hr	96 hr		
0(Control)	0	0	0	0	0	0	0	0
	0	0	0	0	0	0		
0.03	0	0	0	0	0	0	0	0
	0	0	0	0	0	0		
0.06	0	0	0	0	0	0	0	0
	0	0	0	0	0	0		
0.12	0	0	0	0	0	0	0	0
	0	0	0	0	0	0		
0.25	0	0	0	0	0	0	0	0
	0	0	0	0	0	0		
0.50	0	0	0	0	0	0	0	0
	0	0	0	0	0	0		

C. Analysis of LC50

All of the 5 concentrations caused no mortality within the period of the test (LC50 > 0.5 mL/L)

CONCLUSION

According to the Table 2.2, all of the 5 concentrations caused no mortality within the period of the test. The result showed that LC_{50} of the “Novel Juice” was > 0.5 mL/L

REFERENCES

- (一) OECD. 1992. Guideline for Testing of Chemicals, Test No. 203: Fish, Acute Toxicity Test.
- (二) U.S. Environmental Protection Agency. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. 3rd ed. EPA / 600 / 4 – 85 / 013. U.S. EPA Publication, Cincinnati, OH. 1985.