Institute for Environmental Health and Related Product Safety

Chinese Center for Disease Control and Prevention

Test Report

Sample No.: 2011KF0435

Test Item: Goodview™ Nucleic Acid Stain

Sponsor: Beijing SBS Genetech Co., LTD

09/30/2011

The Report of Acute Oral Toxicity Test

Test item: Goodview™ Nucleic Acid Stain

Sample No.: 2011KF0435

Subject title: Acute Oral Toxicity Test

Amount of sample: 300mL

Cat. No.: HGV-II

Sample Trademark: GoodView

Lot No.: 2011.6.8/20110608

Color: Brown-red

Production Unit: Beijing SBS Genetech Co., LTD

Date of sample receipt: 06/09/2011

Date of test complete: 08/02/2011

Sponsor: Beijing SBS Genetech Co., LTD

Address: Room 202, Building 2, No.1 Shangdi 4th Street, Haidian District, Beijing, China

Test criteria: Hygienic Standard for Cosmetics (2007), Technical Specification for Identification of Toxic Chemicals (2005)

I. Material and Method:

1. Sample: Original liquid of GoodView

2. Animals: The animals used in the test were healthy Kunming mice, which were purchased from the National institute for the control of pharmaceutical and biological products The Certificate No. was SCXK (Jing) 2009-0017, SPF level, body weight were from 18g to 22g. Before testing, all animals were fasting but water was ad libitum. All animals were housed in clean animal room (CL). The room temperate was from 18℃ to 22℃ and the relative atmospheric humidity was from 45 to 65%. The Certificate No. of the animal room was SCXK (Jing) 2010-0029.

3. Dose Design: According to Horn's Method, the dosages for the test groups were designed to be 1000, 2150, 4640 and 10000mg/kg. The mice were divided into 4 groups (5animal/sex/group) at random. Animals were administrated with test item by oral gavage, calculate the amount of exposure according to 0.2ml/10g.

4. Outcome Measures: After exposure, general situation, poisoning symptom and death situation were observed, the observed period was two weeks. If there was no mouse die in two weeks, we can decide the LD50 is above 10000mg/kg.

II. Test result:

Results of Acute Oral Toxicity Test with Goodview™ Nucleic Acid Stain

Sex	Dose (mg/kg)	Number of Animals	Dead Number of Animals	Dead Rate (%)
Female	1000	5	0	0
	2150	5	0	0
	4640	5	0	0
	10000	5	0	0
Male	1000	5	0	0
	2150	5	0	0
	4640	5	0	0
	10000	5	0	0

III. Conclusion: After exposure, poisoning symptom was not observed. That means LD_{50} is all 10000mg/kg. Goodview™ Nucleic Acid Stain belongs to nontoxic.

The Report of Mouse marrow chromophilous erythrocyte micronucleus test

Test item: Goodview^{TM} Nucleic Acid Stain

Sample No.: 2011KF0435

Subject title: Mouse marrow chromophilous erythrocyte micronucleus test

Amount of sample: 300mL

Cat. No.: HGV-II

Sample Trademark: GoodView

Lot No.: 2011.6.8/20110608

Color: Brown-red

Production Unit: Beijing SBS Genetech Co., LTD

Date of sample receipt: 06/09/2011

Date of test complete: 09/14/2011

Sponsor: Beijing SBS Genetech Co., LTD

Address: Room 202, Building 2, No.1, Shangdi 4th Street, Haidian District, Beijing, China

Test criteria: Hygienic Standard for Cosmetics (2007), Technical Specification for Identification of Toxic Chemicals (2005)

I. Objective: This test was performed to assess the effect of Goodview™ Nucleic Acid Stain on the incidence of micronuclei of bone marrow polychromatic erythrocytes of the mouse.

II. Material and Animals:

1. Sample: Original liquid of GoodView

2. Positive Control: Mitomycin C, SIGMA ALDRICH.INC 028K1815 C15H18N4O5 M4287

3. Animals: The animals used in the test were healthy Kunming mice, which were purchased from the National institute for the control of pharmaceutical and biological products The Certificate No. was SCXK (Jing) 2009-0017, cleaning level, body weight were from 25g to 30g.

III. Method:

1. Test criteria: Hygienic Standard for Cosmetics (2007), Technical Specification for Identification of Toxic Chemicals (2005)

2. Method: Mice in test groups were administrated orally with the test item twice in 30 hours. The dosages of test groups were 1000, 2000 and 5000mg/kg, respectively. The negative control was administrated with distilled water and the positive control group was administrated with Mitomycin C (1.5mg/kg). In each group, five males and five females mice were used. After 6 hours of the second treatment, the mice were sacrificed. The marrow of sternum was taken and the marrow suspension was made into slides. The slides were stained with Giemsa's and examined under the microscope. 1000 polychromatic erythrocytes (PCE) were observed for each animal. The number of cells with micronucleus was counted.

IV. Test result:

Table 1 Results of Mouse marrow chromophilous erythrocyte micronucleus test with Goodview™ Nucleic Acid Stain

Sex	Group	Dose (mg/kg)	Number of Animals	PCEs	PCEs with micronuclei	The incidences of micronuclei (‰)	*P*
Female	Goodview™ Nucleic Acid Stain	1000	5	5000	4	0.80±0.45	>0.05
		2000	5	5000	4	0.80±0.45	>0.05
		5000	5	5000	3	0.60±0.55	>0.05
	Distilled water	-	5	5000	4	0.80±0.45	
	Mitomycin C	1.5	5	5000	135	27.0±1.22	<0.01
Male	Goodview™ Nucleic Acid	1000	5	5000	4	0.80±0.45	>0.05
		2000	5	5000	4	0.80±0.45	>0.05

	Stain	5000	5	5000	3	0.60±0.55	>0.05
	Distilled water	-	5	5000	3	0.60±0.55	
	Mitomycin C	1.5	5	5000	123	24.6±3.05	<0.01

V. Conclusion: The results showed that there was no significant difference (P>0.05) in the incidence of micronuclei between the test groups and negative control while there was significant difference (P<0.01) between Mitomycin C and negative control. That means the result of Mouse marrow chromophilous erythrocyte micronucleus test with Nucleic Acid Stain is negative.

The Report of Ames Test

Test item: Goodview™ Nucleic Acid Stain

Sample No.: 2011KF0435

Subject title: Ames Test

Amount of sample: 100mL

Cat. No.: HGV-II

Sample Trademark: GoodView

Lot No.: 2011.6.8/20110608

Color: Brown-red

Production Unit: Beijing SBS Genetech Co., LTD

Date of sample receipt: 06/09/2011

Date of test complete: 08/25/2011

Sponsor: Beijing SBS Genetech Co., LTD

Address: Room 202, Building 2, No.1, Shangdi 4th Street, Haidian District, Beijing, China

Test criteria: Hygienic Standard for Cosmetics (2007), Technical Specification for Identification of Toxic Chemicals (2005)

I. Materials and Method

Strains: Salmonella typhimurium strains TA_{97}, TA_{98}, TA_{100} and TA_{102} were identified by our laboratory, the strains were fulfilled the experimental conditions set up by Ames.

Test item: Goodview™ Nucleic Acid Stain is brown-red liquid and can be dissolved in water. The test item was dissolved in sterile distilled water, and the dosages were 0.5, 1.0, 2.5, and 5mg/mL, respectively.

Chemical reagents: 1,8-hydroxyanthraquinone; 2-AF; 9-fluorenone; S9（The S9 mix preparation was performed according to Ames et al and stored in -80℃）, NaN3 and Mitomycin C.

Grouping: The dosages were 0.5, 1.0，2.5 and 5.0mg/plate, respectively. Three parallel plates were set for different dosages. The control groups included blank control plates, solvent control plates (sterile distilled water) and positive control plates. In the absence of S9 mix, the positive control reference substance for strains TA_{97} and TA_{98} was 9-fluorenone, for TA_{100} was NaN3, and for TA_{102} was Mitomycin C. In the presence of S9 mix, the positive control reference substance for strains TA_{97}, TA_{98} and TA_{100} was 2-AF, and for TA_{102} was 1,8- hydroxyanthraquinone.

Method: 0.1mL test solution, 0.1mL bacterial suspension and 0.5mL exogenous metabolic system S9 mix / without S9 mix were mixed uniformly in the test tubes with 1.5mL overlay agar (liquid, 45℃). The mixture was uniformly poured on the prepared underlay agar plates. After solidification, the plates were incubated for 48h at 37℃ in the constant temperature incubator and then the number of revertant colonies per plate was counted. If the number was more than twice the spontaneous revertant colonies counts and showed a dose-response relationship, the positive result could be concluded.

II. Test result

Results of Goodview™ DNA Ames test ($\bar{X}$±SD)

Dose level	TA_{97}		TA_{98}		TA_{100}		TA_{102}	
(mg/plate)	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Blank control	135±12	156±5	30±1	32±1	136±21	155±11	247±10	275±14
Water	132±16	155±2	30±2	32±1	146±10	158±19	248±7	275±15
0.5	142±9	141±14	31±1	32±2	137±17	154±13	261±9	266±10
1	130±5	151±13	30±1	31±2	140±14	168±10	251±8	258±6
2.5	138±13	151±7	30±1	31±1	142±12	167±7	259±13	254±5
5	136±10	159±2	31±2	32±1	152±10	172±28	254±15	282±6
Positive control (ug/plate)								
NaN_3 2.5					1283±26			
2-AF 10.0		1519±161		2306±234		1338±166		
9-Fluorenone 0.2	1475±105		2244±36					
Mitomycin C 4.0							1464±156	
1,8-hydroxyanthraquinone 50.0								1562±285

III. Conclusion: Four strains including Salmonella typhimurium strains TA_{97}, TA_{98}, TA_{100} and TA_{102} were tested by the Test Substance, no matter directly response detection or post-metabolic activation detection. No mutagenicity was observed. According to the guidelines, the result of Ames test with Goodview™ Nucleic Acid Stain is negative.

The Report of in Vitro Mammalian Cell Chromosome Aberration Test

Test item: Goodview™ Nucleic Acid Stain

Sample No.: 2011KF0435

Subject title: in Vitro Mammalian Cell Chromosome Aberration Test

Amount of sample: 300mL

Cat. No.: HGV-II

Sample Trademark: GoodView

Lot No.: 2011.6.8/20110608

Color: Brown-red

Production Unit: Beijing SBS Genetech Co., LTD

Date of sample receipt: 06/09/2011

Date of test complete: 09/09/2011

Sponsor: Beijing SBS Genetech Co., LTD

Address: Room 202, Building 2, No.1, Shangdi 4th Street, Haidian District, Beijing, China

Test criteria: Hygienic Standard for Cosmetics (2007), Technical Specification for Identification of Toxic Chemicals (2005)

I. Materials and Methods:

1. Cell Strains: Chinese hamster ovary line

2. Metabolic Activation System: S9 mix, which is rat liver homogenate induced by both phenobarbital sodium and naphthaflavone and add some appropriate confactors.

3. Test Compound: 5.0mg/ml of MEM stock solution, separately diluted to different concentration by serum-free culture solution(3h, presence and absence of S9 mix) and MEM medium supplemented with 10% fetal bovine serum(24hours, absence of S9 mix).

4. Method:

(1) **Culture solution**: MEM medium supplemented with 10% fetal bovine serum and 100IU/ml of penicillin and streptomycin.

(2) **Maxmium Final Concentration Determination:** It is shown that the metabolic activation way is +S9 and -S9, and the action time are 3 hours through two-time preliminary experiments. We also confirmed the highest final concentration of the -S9 metabolic activation way responding for 24 hours at the two different conditions. Both of the two preliminary experiments contain test group and blank control group. After high-density inoculation 96 well plate with CHO cell, 37℃, 5%CO_2 for 24h,discard the medium in the plate, add test compound with different concentration and serum-free culture solution, add S9 mix into metabolic activation set, only add culture solution into blank control set, and incubate for 3h in the constant temperature incubator. Discard the culture solution, wash cells 3 times with D-hanks, add culture solution supplemented with 10% fetal bovine serum, and continue to incubate for 24h. Measuring cell activity with resazurin, and determine the final concentration according to cell inhibiting rate.

Chromosome aberration test: Two kinds of test condition, one kind of it is metabolic activation is +S9 and –S9, action time is 3h, another one is –S9, action time 24h. ①In the first test condition: test compound group, negative controls and positive control group were established. According to the result of preliminary test, the final concentration of the test compound is 1250.0, 2500.0 and 5000.0ug/ml(-S9), and 1250.0, 2500.0 and 5000.0ug/ml(+S9). Inoculation plate with CHO cell, and inoculation density is 1.2X10^6/plate, 37℃, 5%CO_2 for 24h, discard the medium in the plate, add test compound with different concentration and serum-free culture solution, add S9 mix into metabolic activation set, and incubate for 3h in the constant temperature incubator. ②In the second test condition: inoculation density of CHO cell is 1.0X10^6/plate, 37℃, 5%CO_2 for

24h, discard the medium in the plate, add test compound with different concentration and medium supplemented with 10% fetal bovine serum, and incubate for 24h in the constant temperature incubator.

(3) **Cell Harvesting:** 4h before harvesting, add colchicines to 1.0ug/ml. Hypoton, fixation, tabletting and dye with Giemsa. Select 200 normal metaphase cell in test compound set and blank control set, and select 100 normal metaphase cell in positive control set, then analysis the chromosome aberration. Record the chromosome aberration type and number and figure out the chromosome aberration rate.

(4) **Statistical Analysis:** Making comparison between chromosome aberration rate of each test compound and chromosome aberration rate of negative control set by x^2 analysis.

II. Test result:

Table 1. The results of in Vitro Mammalian Cell Chromosome Aberration Test (3h, -S9)

Group	Final Concentration (ug/ml)	Total Cell Number	Aberrant Cell Number	Aberration Rate (%)
Negative control	-	200	3	1.5
test compound	1250.0	200	2	1.0
	2500.0	200	4	2.0
	5000.0	200	1	0.5
Mitomycin C	1.0	100	11	11.0*

* $P<0.01$

Table 2. The results of in Vitro Mammalian Cell Chromosome Aberration Test (3h, +S9)

Group	Final Concentration (ug/ml)	Total Cell Number	Aberrant Cell Number	Aberration Rate (%)
Negative control	-	200	1	0.5
Test compound	1250.0	200	0	0
	2500.0	200	0	0
	5000.0	200	0	0
Cyclophosphamide	15.0	100	13	13.0*

* $P<0.01$

Table 3 The result of in Vitro Mammalian Cell Chromosome Aberration Test (24h, -S9)

Group	Final Concentration (ug/ml)	Total Cell Number	Aberrant Cell Number	Aberration Rate (%)
Negative control	-	200	5	2.5
Test compound	31.2	200	3	1.5
	62.5	200	2	1.0
	125.0	200	1	0
Cyclophosphamide	1.6	100	14	14.0*

* $P<0.01$

III. Conclusion: Under the experimental conditions, in vitro mammalian cell chromosome aberration detection system, compared to negative control group, no matter add metabolic activation system or not, the test compound didn't lead to higher chromosome aberration rate, so the result of in vitro mammalian cell chromosome aberration test with Nucleic Acid Stain is negative.