November 24, 2015

Test Report

Client TOSHO Co., Ltd.

一般財団法人
日本食品分析センター
52-1 Motoyoyogi-cho, Shibuya-ku,
Tokyo 151-0062, Japan

Sample Environment-friendly multi-purpose grease cleaning detergent

Title Disinfectant Efficacy Test

Results of testing of the above described sample submitted to this center on October 9, 2015 are reported here.

Disinfectant Efficacy Test

1 Client

TOSHO Co., Ltd.

2 Sample

Environment-friendly multi-purpose grease cleaning detergent

3 Purpose of testing

The purpose of this testing is to determine the disinfectant efficacy of the sample detergent.

4 Test summary

The sample solution was injected with a bacterial solution containing E. coli (Serotype O157:H7, verotoxin producing VT1 and VT2), Salmonella or Staphylococcus aureus (hereinafter referred to as “test solution”) and stored at room temperature. The number of viable bacteria was measured after 1, 5, and 15 minutes.

In addition, a preparatory test was carried out and the determination method of viable bacteria count was considered.

5 Test results

Test results are shown in Table 1.

To confirm that the viable bacterial count is not affected by the sample, a preparatory test by diluting the test solution 10 times with SCDLP medium was carried out

Table-1 Results of viable bacterial count in the test solution

Tested bacteria	Target	Viable bacterial count (/mL)			
		Start time*1	after 1 min.	after 5 min.	after 15 min.
E. coli (O157:H7)	Sample*2	4.8×10^5	2.6×10^4	<10	<10
	Control	4.8×10^5	—	—	5.4×10^5
Salmonella	Sample*2	6.9×10^5	80	<10	<10
	Control	6.9×10^5	—	—	7.0×10^5
Staphylococcus aureus	Sample*2	6.9×10^5	<10	<10	<10
	Control	6.9×10^5	—	—	6.6×10^5

Control: Purified water (saline for Staphylococcus aureus)

Storage temperature: Room temperature

<10: not detected

—: not performed

*1 Viable bacterial count of the control at start is determined immediately after injection of the bacterial solution.

*2 A 4 g sample was added to 100 mL purified water, and mixed.

6 Test method

1) Tested bacteria

Escherichia coli ATCC 43895 (E. coli, Serotype O157:H7, verotoxin producing VT1 and VT2)

Salmonella enterica subsp. *enterica* NBRC 3313

Staphylococcus aureus subsp. *aureus* NBRC 12732

2) Medium for bacterial count and culture conditions

SCDLP agar medium (Nihon Pharmaceutical Co., Ltd.), pour plate method, 35℃±1 ℃, for 2 days.

3) Preparation of test bacterial solution

The test bacteria was cultured on a standard agar medium (Eiken Chemical Co., Ltd.) at 35 ℃±1 ℃ for 18-24 hours, and then suspended in purified water (saline for Staphylococcus aureus). Bacterial count was adjusted to 10^7 - 10^8/mL to prepare the test bacterial solution.

4) Test Procedures

To prepare the test solution, 0.1 mL of the test bacterial solution was injected to 10 mL sample solution (A 4 g sample was added to 100 mL purified water and mixed). The test solution was stored at room temperature and after 1, 5 and 15 minutes the solution was promptly diluted 10 times in SCDLP agar medium (Nihon Pharmaceutical Co., Ltd.). Finally, viable bacterial counts of the test liquid were determined using the media for bacterial count.

Furthermore, purified water (saline for Staphylococcus aureus) was tested in the same way, and viable bacterial counts were determined at start time and 15 minutes later.

December 1, 2015

Test Report

Client TOSHO Co., Ltd.

一般財団法人
日本食品分析センター
52-1 Motoyoyogi-cho, Shibuya-ku,
Tokyo 151-0062, Japan

Sample Environment-friendly multi-purpose grease cleaning detergent

Title Acute oral toxicity test using female mice

Results of testing of the above described sample submitted to this center on October 9, 2015 are reported here.

Acute oral toxicity test using female mice

Summary

An acute oral toxicity test using female mice (limit test) was carried out with the environment-friendly multi-purpose grease cleaning detergent set as the sample.

A 4 g sample was added to 100 mL injection solvent and mixed, and the test solution was given as a single oral administration to female mice at a dosage of 20 mL/kg, and then observed for 14 days.

As a result, no case of abnormality or fatality was observed during the observation period. According the above, for a single oral administration using mice, the LD_{50} value of the test solution was evaluated as exceeding 20 mL/kg in females.

1 Client

TOSHO Co., Ltd.

2 Sample

Environment-friendly multi-purpose grease cleaning detergent

3 Test implementing facility

Japan Food Research Laboratories Tama Laboratory

11-10 Nagayama 6-chome, Tama-shi, Tokyo 206-0025, Japan

4 Test period

From October 9, 2015 to December 1, 2015

5 Purpose of testing

A 4 g sample was added to 100 mL injection solvent, and mixed. Acute oral toxicity of this test solution in female mice was considered in compliance with the OECD Guidelines for the Testing of Chemicals 420 (2001).

6 Preparation of test solution

A 2 g sample was dissolved in 10 mL of injection solvent which was heated to approx. 40 ℃. 40 mL of injection solvent was added, and this was set as the test solution.

The injection solvent which was heated to approx. 40 ℃ and left at room temperature was set as the blank test solution.

7 Test animals

5 week old ICR female mice were purchased from Japan SLC, Inc. and used for the test approx. after 1 week of preparatory raising (quarantine) to confirm no abnormalities under normal conditions. The test animals were kept in polycarbonate cages in sets of 5 animals, and raised in a breeding house with a room temperature of 23℃±3℃ and lighting 12 hours/day. The mice were allowed to freely eat [solid feed for mice, rats: Labo MR Stock, Nosan Corporation] and drink (tap water).

8 Test method

5 mice were set as a test group. The test group was administered 20 mL/kg as the administration dose of the test solution. 5 mice were set as a control group and were administered a blank test solution.

The test animals were fasted approx. 4 hours before administration. After measuring body weight, 20 mL/kg of test solution was administered to the test group as a forced single oral dose using a stomach tube. 20 mL/kg of the blank test solution was administered to the control group in the same manner.

14 days were set as the observation period, and observation was carried out frequently on the administration day, and once 1 day from the following day onward. Body weight was measured on 7th and 14th day after administration, and Levene's test was carried out. No difference in dispersion was observed, so comparison between groups was carried out by Student's t-test. Level of significance was set at 5 %. Autopsy was carried out on all the animals after the observation period was completed.

9 Test results

1) Fatality cases

No case of fatality was observed during the observation period in either administration group.

2) Normal condition

No abnormalities were observed during the observation period in either administration group.

3) Body weight change (Table-1)

No change in body weight value was observed in test group on 7th and 14th day after administration, compared to the control group.

4) Autopsy findings

An autopsy was performed after the observation period was completed. No abnormalities were observed in any of the animals.

10 Conclusion

An acute oral toxicity test (limit test) of the test solution was carried out using female mice.

As a result, no case of abnormality or fatality was observed during the observation period. According to the above, the LD_{50} value of the test solution was evaluated as exceeding 20 mL/kg in females for a single oral administration using mice.

Table-1 Body weight change

Administration group	Before administration	After administration (day)	
		7	14
Test group	27.6±0.9 (5)	29.7±1.2 (5)	31.6±1.5 (5)
Control group	27.6±0.8 (5)	29.3±1.2 (5)	31.7±1.4 (5)

The body weight is shown as the average value ± standard deviation (unit: g).

Number of test animals is shown in the brackets.

December 1, 2015

Test Report

Client TOSHO Co., Ltd.

一般財団法人
日本食品分析センター
52-1 Motoyoyogi-cho, Shibuya-ku,
Tokyo 151-0062, Japan

Sample Environment-friendly multi-purpose grease cleaning detergent

Title Primary skin irritation test using rabbits

Results of testing of the above described sample submitted to this center on October 9, 2015 are reported here.

Primary skin irritation test using rabbits

Summary

A primary skin irritation test using rabbits was carried out in compliance with the OECD Guidelines for the Testing of Chemicals 404 (2002), with the environment-friendly multi-purpose grease cleaning detergent set as the sample.

A 4 g sample was added to 100 mL injection solvent and mixed, and then applied and covered for 4 hours on the uninjured and/or injured skin of 3 rabbits. As a result, clear erythema was observed in all cases one hour after removal, but disappeared within 48 hours.

Primary irritation index (P.I.I.) was calculated according to the ISO 10993-10 Biological Evaluation of Medical Devices–Part 10 (2010) was 0.3.

According to the above, in the primary skin irritation test using rabbits, the test solution was evaluated as being within the range of "nonstimulating."

1 Client

TOSHO Co., Ltd.

2 Sample

Environment-friendly multi-purpose grease cleaning detergent

3 Test implementing facility

Japan Food Research Laboratories Tama Laboratory

11-10 Nagayama 6-chome, Tama-shi, Tokyo 206-0025, Japan

4 Test period

From October 9, 2015 to December 1, 2015

5 Purpose of testing

A 4 g sample was added to 100 mL injection solvent and mixed. Primary skin irritation was considered for this test solution using rabbits, in compliance with the OECD Guidelines for the Testing of Chemicals 404 (2002).

6 Preparation of test solution

A 2 g sample was dissolved in 10 mL of the injection solvent which was heated to approx. 40 ℃. 40 mL of injection solvent was then added, and this was set as the test solution.

7 Test animals

Male Japanese white rabbits were purchased from Kitayama Labes Co., Ltd. Three animals were used for the testing after more than 1 week of preparatory raising (quarantine) to confirm no abnormalities in general condition. The test animals were kept in FPR cages individually, and raised in a breeding house with a room temperature of 23 ℃±3 ℃ and lighting 12 hours/day. Solid feed for rabbits and guinea pigs [LRC4, Oriental Yeast Co., Ltd.] were limited supplied, and tap water was freely provided.

8 Test method

Hair on the back of the each test animal was shaved approx. 24 hours before the test.

For each animal, four locations in approx. 6 cm^2 were set. In two locations, the skin was scratched on the keratinized layer (injured skin) in a double cross shape without reaching the dermis using an 18 gauge injection needle. The other two locations were set as non-treated (uninjured skin) areas.

0.5 mL test solution was uniformly coated on a gauze patch, which was cut to approx. 2 cm × 3 cm and applied to one location each of the uninjured and injured skin, and secured with multi-fix roll [ALCARE Co., Ltd.]. In addition, to ensure that the patch was in contact with the skin, Blenderm™ surgical tape [3M Health Care] was applied. The remaining uninjured and injured locations of the skin were set as control.

Application time was set at 4 hours, and the patches were removed after that. The applied site was then cleaned with the injection solution. After removal, observation was carried out after 1, 24, 48, and 72 hours, and scoring of irritation reaction was carried out according to Table-1.

Moreover, following ISO 10993-10 Biological Evaluation of Medical Devices–Part 10 (2010), scores at 24, 48, and 72 hours after patch removal were totaled and then divided by 6, and the average of each test animal was calculated as the primary irritation index (P.I.I.), and irritation of the test solution, based on the standard shown in Table-2, was evaluated.

Furthermore, body weight of the test animals was measured at the beginning and end of the test.

9 Test results (Table-3 and 4)

1) Test animals ①

Clear erythema (score 2) was observed on the uninjured and injured skin one hour after removal, but became mild erythema (score 1) after 24 hours, and disappeared within 48 hours. Afterwards, no irritation reaction was observed.

2) Test animals ②

Clear erythema (score 2) was observed on the uninjured and injured skin 1 hour after removal, but disappeared within 24 hours on the injured skin and became mild erythema (score 1) on the uninjured skin. Erythema on the uninjured skin disappeared within 48 hours, and afterwards, no irritation reaction was observed.

3) Test animals ③

Clear erythema (score 2) was observed on the uninjured and injured skin 1 hour after removal, but became mild erythema (score 1) after 24 hours, and disappeared within 48 hours. Afterwards, no irritation reaction was observed.

P.I.I. calculated according to the scoring results was 0.3.

Furthermore, no irritation reaction was observed on the uninjured skin of the non-treated area or the injured skin of all test animals throughout the observation period.

10 Conclusion

A primary skin irritation test using rabbits was carried out for this test solution in compliance with the OECD Guidelines for the Testing of Chemicals 404 (2002).

As a result, clear erythema was observed in all cases one hour after removal, but disappeared within 48 hours.

Primary irritation index (P.I.I.) was calculated according to the ISO 10993-10 Biological Evaluation of Medical Devices–Part 10 (2010) was 0.3.

According to the above, in the primary skin irritation test using rabbits, the test solution was evaluated as being within the range of "nonstimulating."

11 References

• ISO 10993-10 Biological Evaluation of Medical Devices–Part 10: Tests for irritation and skin sensitization (2010).

Table-1 Evaluation of skin reaction

Formation of erythema and crusts

No erythema ····· 0

Very mild erythema (barely identified) ····· 1

Clear erythema ····· 2

Midrange to severe erythema ····· 3

From severe erythema (dark red) to the formation of crusts which hinder the scoring of erythema ····· 4*

[Highest score: 4]

*Reactions such as necrosis, ulcer, loss of hair, and scar are classified as deep damage with a score of 4.

Edema formation

No edema ····· 0

Very mild edema (barely identified) ····· 1

Mild edema (clear edge due to swelling can be identified) ····· 2

Midrange edema (approx. 1 mm swelling) ……3

Severe edema (swelling greater than 1 mm and spreading beyond exposed range) ·4

[Highest score: 4]

Table-2 Categories of primary irritation reaction in rabbits

Categories of reaction	P.I.I.
Nonstimulating	0 - 0.4
Mild irritation	0.5 - 1.9
Midrange irritation	2 - 4.9
Severe irritation	5 - 8

Table-3 Body weight of the test animals (unit: kg)

Test animals	At the start of the test	At the end of the test
①	3.30	3.30
②	3.00	3.08
③	3.07	3.16

Table-4 Scoring results of skin reaction

Observation time	Test animals ①		Test animals ②		Test animals ③	
	Uninjured	Injured	Uninjured	Injured	Uninjured	Injured
1 hour	2/0	2/0	2/0	2/0	2/0	2/0
24 hours	1/0	1/0	1/0	0/0	1/0	1/0
48 hours	0/0	0/0	0/0	0/0	0/0	0/0
72 hours	0/0	0/0	0/0	0/0	0/0	0/0

Results are shown in the order of erythema, crusts and edema.

December 1, 2015

Test Report

Client TOSHO Co., Ltd.

一般財団法人
日本食品分析センター
52-1 Motoyoyogi-cho, Shibuya-ku,
Tokyo 151-0062, Japan

Sample Environment-friendly multi-purpose grease cleaning detergent

Title Eye irritation test using rabbits

Results of testing of the above described sample submitted to this center on October 9, 2015 are reported here.

Eye irritation test using rabbits

Summary

An eye irritation test using rabbits was carried out in compliance with the OECD Guidelines for the Testing of Chemicals 405 (2012), with the environment-friendly multi-purpose grease cleansing detergent set as the sample.

A 4 g sample was added to 100 mL injection solvent and then mixed. 0.1 mL was dripped in one eye each of 3 rabbits, and 0.1 mL injection solvent as the control solution was dripped in the other eye. As a result, no irritation reaction was observed in either the test eye or the control eye, for each observation time of 1, 24, 48, and 72 hours after adding the drops.

The highest value obtained as the average total score for both tested eye and control eye during the observation period, as calculated according to the Draize method, was 0.

According the above, in the eye irritation test using rabbits, the test liquid was evaluated as being within the range of “nonstimulating”.

1 Client

Tosho Co., Ltd.

2 Sample

Environment-friendly multi-purpose grease cleaning detergent

3 Test implementing facility

Japan Food Research Laboratories Tama Laboratory

11-10 Nagayama 6-chome, Tama-shi, Tokyo 206-0025, Japan

4 Test period

From October 9, 2015 to December 1, 2015

5 Purpose of testing

A 4g sample was added to 100 mL injection solvent, and mixed. Eye irritation was considered for this test solution using rabbits, in compliance with the OECD Guidelines for the Testing of Chemicals 405 (2012).

6 Preparation of test solution

A 2 g sample was dissolved in 10 mL of the injection solvent which was heated to approx. 40 ℃. 40 mL of injection solvent was then added, and this was set as the test solution.

The injection solvent which was heated to 40 ℃ and left at room temperature was set as the blank test solution.

7 Test animals

Male Japan white rabbits were purchased from Kitayama Labes Co., Ltd. 3 animals were used for the testing after more than 1 week of preparatory raising (quarantine) to confirm no abnormalities in general condition. The test animals were kept in FPR cages individually, and raised in a breeding house with a room temperature of 23 ℃±3 ℃ and lighting 12 hours/day. Solid feed for rabbits and guinea pigs [LRC4, Oriental Yeast Co., Ltd.] were limited supplied, and tap water was freely provided.

8 Test method

The anterior part of both eyes of each test animal was tested on the first day of the test, to confirm that there was no abnormality.

After measuring body weight, 0.1 mL of the test solution was dropped on conjunctival sac of one eye of each test animals, and the top and bottom eye lid were gently closed and preserved for approx one second. Blank test solution was dropped in the other eye as the control solution. 1, 24, 48, and 72 hours after adding the drops, the cornea, iris, conjunctiva, etc., were observed using a slit lamp (×10) [Ohira Co., Ltd.], and the degree of eye irritation was graded according to standards in the Draize method as shown in Table 1.

Furthermore, occurrence of corneal epithelium disorder or not and its degree were observed in detail using sodium fluorescein, at each observation time except 1 hour after adding the drops.

The total score of each animal was calculated using the obtained scores, according to the formula shown in Table-2, and the average score of the 3 animals was calculated at each observation time. Eye irritation of test solution was evaluated according the highest value of the average total score during the observation period, according the standard as shown in Table-3.

9 Test results (Table-4 to 8)

1) Test animals ①

No irritation reaction was observed in either the test eye or control eye during the observation period.

Moreover, when the test eye and control eye were tested using sodium fluorescein, no staining was observed for all observation periods.

2) Test animals ②

No irritation reaction was observed in either the test eye or control eye during the observation period.

Moreover, when the test eye and control eye were tested using sodium fluorescein, no staining was observed for all observation periods.

3) Test animals ③

No irritation reaction was observed in either the test eye or control eye during the observation period.

Moreover, when the test eye and control eye were tested using sodium fluorescein, no staining was observed for all observation periods.

The highest value of the average total score, for both the test eye and the control eye during the observation period, was 0.

10 Conclusion

An eye irritation test using rabbits was carried out for this test solution in compliance with the OECD Guidelines for the Testing of Chemicals 405 (2012).

As a result, no irritation reaction was observed on either the test eye or the control eye, for each observation time of 1, 24, 48, and 72 hours after adding the drops.

The highest value obtained as the average total score for both tested eye and control eye during the observation period, as calculated according to the Draize method, was 0.

According to the above, in the eye irritation test using rabbits, the test solution was evaluated as being within the range of “nonstimulating.”

11 References

- “Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics” (1959)
  The Association of Food and Drug Officials of the United States.

Table-1 Evaluation of eye disturbance

(1) Cornea

(A) Degree of opacity (judgement of the thickest area)

Clear, no opacity…… 0

Scattered, diffused opacity, details of the iris can clearly be observed …. 1

Translucent and easily identified, details of the iris are slightly unclear … 2

Milky, pattern of the iris cannot observed, size of pupil can barely be observed 3

Cloudy, iris cannot be observed …… 4

(B) Dimensions of the cornea opacity section (S)

$0 < S \leq 1/4$ …… 1

$1/4 < S \leq 1/2$ …… 2

$1/2 < S \leq 3/4$ …… 3

$3/4 < S \leq 4/4$ …… 4

[Score＝A×B×5 Highest score……80]

(2) Iris

(A) Normal …… 0

One or more of the following- abnormal fold, congestion, enlargement, and/or circumcorneal hyperemia,is observed, but there is light reflection at least …… 1

One or more of the following- no light reflection, bleeding, and significant tissue disruption is observed …… 2

[Score＝A×5 Highest score……10]

(3) Conjunctiva

(A) Reddening of the palpebral conjunctiva and bulbar conjunctiva

Blood vessels are normal …… 0

Clear congestion of blood vessels …… 1

Diffused, crimson color, individual vessels are hard to identify …… 2

Diffused, beef-like red …… 3

(B) Edema of conjunctiva

No enlargement …… 0

Some enlargement (including nictitating membrane) …… 1

Clear enlargement, eyelid is slightly ectropic …… 2

Enlargement, eyelid is half-closed …… 3

Enlargement, eyelid is more than half-closed …… 4

(C) Secretion

Not observed…… 0

Slightly observed …… 1

Secretion that wets the eyelid and the immediately surrounding hair…… 2

Secretion that wets the eyelid and much of the surrounding hair …… 3

[Score＝(A+B+C)×2 Highest score……20]

Table-2 Calculation method of total score

Site	Calculation formula	The highest score
(1) Cornea	A×B×5	80
(2) Iris	A×5	10
(3) Conjunctiva	(A+B+C)×2	20
(1) + (2) + (3)＝Total score*		110

A, B, and C shows the score of (A), (B), and (C) in Table-1.

*Calculated at each observation time.

Table-3 Evaluation of eye irritation

The highest value of the average total score	Classification
0 - 5.0	Nonstimulating compound
5.1 - 15.0	Mildly stimulating compound
15.1 - 30.0	Stimulating compound
30.1 - 60.0	Mid-range stimulating compound
60.1 - 80.0	Mid to severe stimulating compound
80.1 - 110.0	Severe stimulating compound

Table-4 Body weight of the test animals(unit: kg)

Test animals	Body weight(at the start of the test)
①	3.04
②	3.17
③	3.02

Table-5 Change in total score over time

Test animals	Total score at each observation time			
	1 hour	24 hours	48 hours	72 hours
①	0(0)	0(0)	0(0)	0(0)
②	0(0)	0(0)	0(0)	0(0)
③	0(0)	0(0)	0(0)	0(0)
Average total score	0(0)	0(0)	0(0)	0(0)

Results of the control eye are shown in brackets.

Table-6 Score results of the test animals ①

Observation site		Score results			
		1 hour	24 hours	48 hours	72 hours
(1) Cornea	Degree of opacity (A)	0(0)	0(0)	0(0)	0(0)
	Dimensions of the opaque area (B)	-(-)	-(-)	-(-)	-(-)
(2) Iris	(A)	0(0)	0(0)	0(0)	0(0)
(3) Conjunctiva	Reddening (A)	0(0)	0(0)	0(0)	0(0)
	Edema (B)	0(0)	0(0)	0(0)	0(0)
	Secretion (C)	0(0)	0(0)	0(0)	0(0)
Score (1)＝	A×B×5	0(0)	0(0)	0(0)	0(0)
Score (2)＝	A×5	0(0)	0(0)	0(0)	0(0)
Score (3)＝	(A+B+C)×2	0(0)	0(0)	0(0)	0(0)
Total score	[(1)+(2)+(3)]	0(0)	0(0)	0(0)	0(0)

Results of the control eye are shown in brackets.

-: Not judged

Table-7 Score results of the test animals ②

Observation site		Score results			
		1 hours	24 hours	48 hours	72 hours
(1) Cornea	Degree of opacity (A)	0(0)	0(0)	0(0)	0(0)
	Dimensions of the opaque area (B)	-(-)	-(-)	-(-)	-(-)
(2) Iris	(A)	0(0)	0(0)	0(0)	0(0)
(3) Conjunctiva	Reddening (A)	0(0)	0(0)	0(0)	0(0)
	Edema (B)	0(0)	0(0)	0(0)	0(0)
	Secretion (C)	0(0)	0(0)	0(0)	0(0)
Score (1)=	A×B×5	0(0)	0(0)	0(0)	0(0)
Score (2)=	A×5	0(0)	0(0)	0(0)	0(0)
Score (3)=	(A+B+C)×2	0(0)	0(0)	0(0)	0(0)
Total score	[(1)+(2)+(3)]	0(0)	0(0)	0(0)	0(0)

Results of the control eye are shown in brackets.

-: Not judged

Table-8 Score results of the test animals ③

Observation site		Score results			
		1 hours	24 hours	48 hours	72 hours
(1) Cornea	Degree of opacity (A)	0(0)	0(0)	0(0)	0(0)
	Dimensions of the opaque area (B)	-(-)	-(-)	-(-)	-(-)
(2)Iris	(A)	0(0)	0(0)	0(0)	0(0)
(3) Conjunctiva	Reddening (A)	0(0)	0(0)	0(0)	0(0)
	Edema (B)	0(0)	0(0)	0(0)	0(0)
	Secretion (C)	0(0)	0(0)	0(0)	0(0)
Score (1)=	A×B×5	0(0)	0(0)	0(0)	0(0)
Score (2)=	A×5	0(0)	0(0)	0(0)	0(0)
Score (3)=	(A+B+C)×2	0(0)	0(0)	0(0)	0(0)
Total score	[(1)+(2)+(3)]	0(0)	0(0)	0(0)	0(0)

Results of the control eye are shown in brackets.

-: Not judged

December, 4, 2015

Test Report

Client TOSHO Co., Ltd.

一般財団法人
日本食品分析センター
52-1 Motoyoyogi-cho, Shibuya-ku,
Tokyo 151-0062, Japan

Sample Environment-friendly multi-purpose grease cleaning detergent

Title Biodegradability test by DOC method

Results of testing of the above described sample submitted to this center on October 9, 2015 are reported here.

Biodegradability test by DOC method

Summary

A biodegradability test was carried out on the sample for 14 days, in compliance with the OECD Guidelines for the Testing of Chemicals 301A (1992).

For the test, supernatant solution and secondary treated water of standard activated sludge was used, and cultured by the shake culture method. Dissolved organic carbon (hereinafter abbreviated as to "DOC") was measured.

As a result of the test, biodegradability level of the sample after 14 days was 84%.

Client

TOSHO Co., Ltd.

Sample

Environment-friendly multi-purpose grease cleaning detergent

Test period

From October 9, 2015 to December 4, 2015

Test implementing facility

Japan Food Research Laboratories Tama Laboratory

11-10 Nagayama 6-chome, Tama-shi, Tokyo 206-0025, Japan

Person in charge of testing

Japan Food Research Laboratories Tama Laboratory

Safety Test Department, Safety Test Division

Yuuji Yoshiyasu

1 Purpose of testing

The purpose of the testing is to measure biodegradability of the sample.

2 Sample

Environment-friendly multi-purpose grease cleaning detergent

3 Test method

1) Test classification

① Culture test section: sample + microbe source + basic medium (Test frequency: 3)

② Non-culture test section: sample + water + bactericidal agent

③ Adsorption test section: sample + microbe source + basic medium+ bactericidal agent

④ Standard test section: aniline + microbe source + basic medium

⑤ Seeding blank: microbe source + basic medium

2) Study conditions

① Test method: shake culture method (amplitude 10 cm, shake frequency 100 times/min)

② Test period: 14 days (measurement point: at start, 7 and 14 days later)

③ Sample concentration: 300 mg/L (40 mg/L as DOC)

④ Standard substances: aniline [Kanto Chemical Co., Ltd., Prime class, more than 99.0 % purity]

⑤ Standard substance concentration: 50 mg/L (40 mg/L as DOC)

⑥ Microorganism source: supernatant solution and secondary treated water of standard activated sludge

⑦ Microbe source concentration: 20 mL/400 mL

⑧ Basic medium: organic medium

⑨ Culture solution volume: 400 mL

⑩ Test container: 500 mL volume shaker flask

⑪ Test temperature: 22 ℃±2 ℃

3) Preparation of test culture solution and basic medium

① Culture test section, non-culture test section, and adsorption test section

The sample was added to the basic medium or water to become 40 mg/L as DOC, and set as the culture test section, non-culture test section, and adsorption test section. Moreover, 1 mL of 2 W/V% mercuric chloride solution was added to 500 mL in the non-culture test section, and adsorption test section, for sterilization.

② Standard test section

The standard substance (aniline) was added to the basic medium to become 40 mg/L as DOC, and set as the culture test section.

③ Basic medium

The basic medium was prepared according the OECD Guidelines for the Testing of Chemicals 301A (1992).

4) Microbe source

① Standard activated sludge

Obtained from: Chemicals Evaluation and Research Institute, Japan

Date obtained: October 21, 2015

② Secondary treated water

Collected from: Kita-Tama Ichigo Water Reclamation Center, Bureau of Sewage, Tokyo (Fuchu city, Tokyo)

Date collected: October 22, 2015

③ Seeding

After filtering the supernatant solution and secondary treated water of standard activated sludge with a paper filter (No.5A [Toyo Roshi Kaisha, Ltd.]), they were mixed at a ratio of 1:2. This solution was seeded in the culture test section, absorption test section, standard test section, and seeding blank.

Furthermore, seeding ratio was set at 20 mL/400 mL.

5) Measurement of DOC

At start (right after starting and 2 hours later) and 7 and 14 days after starting, the culture solution of each test section was centrifuge separated (4000 g, 15 min.), and the DOC of the supernatant solution was measured using a TOC device.

6) Calculation method of biodegradability

Biodegradability by DOC was calculated using the following formula. However, the non-culture test section was calculated without subtracting the seeding blank.

Furthermore, a decreasing tendency of DOC after 2 hours compared DOC right after starting was observed in the culture test section. This was not biodegradation, but rather was assumed to have been caused by absorption to the test container. Hence, DOC after two hours was used to calculate the test sections except for standard test section.

$$\text{Biodegradability}(\%) = \frac{(T_0 - B_0) - (T_x - B_x)}{(T_0 - B_0)} \times 100$$

T_0: DOC at the start of each test section (mgC/L)

B_0: DOC at the start of the seeding section (mgC/L)

T_x: DOC after x days of each test section (mgC/L)

B_x: DOC after x days of the seeding blank (mgC/L)

7) Measurement device

TOC device: TOC-L [Shimadzu Corporation]

4 Test results

1) Test of biodegradability by DOC method

Biodegradability of the sample and standard substance was shown in Table-1.

The biodegradability level of the sample after 14 days was 84 %. Moreover, the biodegradability level of the standard substance after 14 days was 93 %.

Table-1 Biodegradability of the sample and standard substance (Unit: %)

Test classification	after 7 days	after 14 days	Average value
Sample			
Culture test section 1	86	83	84*
Culture test section 2	86	85	
Culture test section 3	84	85	
Non-culture test section	<5	<5	—
Absorption test section	<5	<5	—
Aniline			
Standard test section	95	93	—

* The average values after 14 days in culture test sections 1-3 are shown.

2) DOC value

DOC values of the sample and standard substance are shown in Table-2.

Furthermore, results except for non-culture test section are shown as value minus seeding blank.

Table-2 DOC value of the sample and standard substance (Unit: mgC/L)

Test classification	At start		7 days after	14 days after
	Right after	2 hours later		
Sample				
Culture test section 1	40.2	37.8	5.3	6.3
Culture test section 2	39.8	38.3	5.5	5.8
Culture test section 3	38.9	37.5	5.9	5.7
Non-culture test section	46.5	42.6	41.3	46.9

Absorption test section	43.0	41.0	39.0	45.1
Aniline				
Standard test section	44.4	43.8	2.3	2.9