Toxicity Test Report

Sample name: 4,4'-METHYLENE-BIS-(ORTHO-CHLOROANILINE)

(MOCA)

Test items: acute oral and dermal toxicity tests

dermal and ophthalmic irritability tests

Guinea pig skin allergy（sensitization）test

Sample unit: Suzhou Xiangyuan Special Fine Chemical Co, Ltd

Test unit: Department of Toxicology

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Catalogue

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Abstract

1. **Test based on:《Technological Specification for Assessment of Chemical Toxicity－2005》**
2. **Test items: MOCA**

   acute oral and dernal toxicity tests

   dermal skin and eye irritant tests

   dermal allergy（sensitization）test
3. **Test results:**

   1) **Rats acute toxicology test:**

      Acute oral LD_{50} was greater than 5000mg/kg for female and male

      acute dermal LD_{50} was greater than 5000mg/kg for female and male

   2) **The dermal and eye irritant test of rabbit:**

      Dermal irritant test: the average accumulated score was 0.

      Eye irritant test(without washing): I.A.O.I is 2－4 and M.I.O.I of 48h was 0. The experimental eyes recovered after 48h of administration.

      3) Guinea pig dermal allergy（sensitization）test: Rate of sensitization was 0, magnitude of sensitization was I.
4. **Conclusions**

   1) **Rat acute toxicity: oral and dermal were belong to the actual no toxicity chemical**

   1) **The sample is not irritant to the dermal and eye of rabbit.**

   2) **MOCA belong to weak-sensitizer.**

MOCA was provided by Suzhou Xiangyuan Special Fine Chemical Co, Ltd. To protect personnels against adverse effect. The toxicity tests of the product submitted to the company were done by our department. According to 《Technological Specification for Assessment of Chemical Toxicity—2005》, acute oral and dermal toxicity tests, dermal and ophthalmic irritability tests, and dermal sensibilization test were made. Results as following:

I the rat acute oral toxicity test

1. materials and methods

1.1 objective: pallide-flavens powder, no obvious smell.

1.2 test animals: Wistar rats, body weight range was180-220 grams, animal test number: 070101, provided by experimental animal center of Shanxi Medical University and fed with standard animal feeds, drinking water freely.

1.3 test groups: test rats were divided randomly into five groups according to weight, eight rats each group, four males and four females every groups.

1.4 test doses: 464mg/kg、1000 mg/kg、2150 mg/kg、4640 mg/kg、5000mg/kg

1.5 test steps: (Single oral feeding) . The mixture of chemical add to animal feeds, according to above test doses, were grinded uniform. Test animals feed and water freely and with mono-cage. Observe toxic symptom for 2 weeks and record toxic symptom and death time. Test animals were provided with normal feeds after next day. The results were computed using Horn method and evaluated according to "acute oral toxicity ranking standard".

2. results

No remarkable anomaly change was found in each group after giving chemical, the animals' drink and food, activity were all normal. Datas were as following(table 1):

Table 1 the acute oral toxicity test results

animal	dose (mg/kg)	Animal numbers	Death numbers	LD_{50} (mg/kg)
female	464	4	0	>5000
	1000	4	0	
	2150	4	0	
	4640	4	0	

	5000	4	0	
male	464	4	0	>5000
	1000	4	0	
	2150	4	0	
	4640	4	0	
	5000	4	0	

3.conclusions: rat oral LD_{50} was greater than 5000mg/kg for male and female, belong to the actual no toxicity chemical.

II the rat acute dermal toxicity test

1. materials and methods

1.1 objective: the same as the objective described in the rat acute toxicity test .

1.2 test animals: the same as the test animals described in the rat acute toxicity test .

1.3 test groups: test rats were divided into five groups according to weight, eight rats each group, four males and four females every groups.

1.4 test doses: 464mg/kg、1000 mg/kg、2150 mg/kg、4640 mg/kg、5000mg/kg

1.5 test steps: Test rats were depilated in the abdomen using 8% sodium sulfide for about $5\times4cm^2$ area. No skin injury was observed after 24h of depilation. Chemical was applied on the depilated area covered with 4 layer bandage by 1ml chemical /100g weight. pasting area of $3\times4cm^2$. The skin was cleaned with warm water after 4h of administration. Observe toxic symptom for 2 weeks and record toxic symptom and death time. Test animals were provided with normal feeds after next day. The results were computed using Horn method and evaluated according to "acute dermal toxicity ranking standard".

2.results

After administration per cutem, no remarkable anomaly change was found, the animals' drink and food, activity were all normal. Datas were as following(table 2):

3.conclusions: rats dermal LD_{50} was greater than 5000mg/kg for male and female, belong to the actual no toxicity chemical.

Table 2 the acute dermal toxicity test results

animal	dose（mg/kg）	Animal numbers	Death numbers	LD_{50} （mg/kg）
female	464	4	0	>5000
	1000	4	0	
	2150	4	0	
	4640	4	0	
	5000	4	0	
male	464	4	0	>5000
	1000	4	0	
	2150	4	0	
	4640	4	0	
	5000	4	0	

III The dermal and eye irritant test of rabbit

1. Meterials and methods

1.1 objective: the same as the objective described in the rat acute toxicity test .

1.2 test animal: rabbit, body weight range was 2.5-3.0kg, provided by experimental animal center of Shanxi Medical University and fed with feeds, drinking water freely.

1.3 rabbit dermal irritant test

1.3.1 test dose: 0.5g per administration

1.3.2 test steps: Four healthy rabbits without skin infections were depilated by 8% sodium sulfide for about $5\times6cm^2$ area. If no skin injury was observed after 24h of depilation, apply 0.5g chemical blended with some distilled water on one side of depilated area and covered with 4 layer bandage and 1 layer glass paper fixed with rubber cement and bandage; the other side of depilated area for control was applied with the same volume of normal sodium. The skin was washed with warm water after 4h of administration.

1.3.3 Observation and evaluation: Observe the skin reaction after 4,24,48,72h. The results were evaluated according to the criterions as *technological specification of chemicals toxicity identification, evaluation on skin irritant reaction, evaluation criterion of skin stimulus intensity.*

1.3Rabbit eye irritant test

1.3.1 Test dose: 0.1g per administration

1.3.2 Test steps: Four healthy rabbits without eye infections were selected. Add 0.1g chemical into

saccuses conjunctivae of the right eyes and gently pressed the internal canthuses for 1 second and washed it after 24h of administration. The left control eyes were added with 0.1ml normal sodium. Observe and record the eye reaction in 1, 24, 48, 72h, 4d and 7d. If the irritant reaction were not disappeared after 72h, add 0.1g chemical into the saccus conjunctivaes of another four rabbits' right eyes ,gently pressed the internal canthuses for 1 second, washed for 5min after 4s of administration then stopped for 30s before another 5min washing.

1.3.3 Observation and evaluation: Observe the eye reaction after 1, 24, 48, 72h, 4d and 7d of treatment and observe the cornea injury in the condition of adding fluorescein sodium into the experimental eyes. The evaluation score were made on the basis of technological specification of chemicals toxicity identification and classification criterion of eye injury and the evaluation conclusion were drawn according to *the evaluation criterion of eye stimulus intensity*.

2.Results

2.1 Rabbit dermal irritant test: Data were as following (table3).

Table 3 the rabbit dermal irritant test results

No.	gender	weight	4h				24h				48h				72h			
		(kg)	S		C		S		C		S		C		S		C	
			E	D	E	D	E	D	E	D	E	D	E	D	E	D	E	D
1	♀	2.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	♀	2.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	♂	2.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	♂	2.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average accumulated points			0				0				0				0			
stimulus intensity			No stimulation				No stimulation				No stimulation				No stimulation			

Note: 1.S and C stands for "sample" and "control".

2.E and D are the first letters of "erythema" and "dropsy".

Summary: The sample is not irritant to the dermal of rabbit (0～0.4).

2.2 Rabbit eye irritant test: See the table 4. Administration without washing: I.A.O.I was 2-4 and M.I.O.I of 48h was 0. The experimental eyes recovered after 48h of administration. See the detail in table 4.

Table 4. the eye irritant test results (without washing)

objective	Eye irritant reaction accumulated point												I.A.O.I		M.I.O.I		stimulus intensity	
	1h		24h		48h		72h		4d		7d							
No.&position	S*	C*	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
1.conjunctiva	2	0	0	0	0	0	0	0	0	0	0	0						
iris	0	0	0	0	0	0	0	0	0	0	0	0			48h			
cornea	0	0	0	0	0	0	0	0	0	0	0	0	2	0	later is	0	(-)	(-)
Total score	2	0	0	0	0	0	0	0	0	0	0	0			0			
2.conjunctiva	2	0	0	0	0	0	0	0	0	0	0	0						
iris	0	0	0	0	0	0	0	0	0	0	0	0			48h			
cornea	0	0	0	0	0	0	0	0	0	0	0	0	2	0	later is	0	(-)	(-)
Total score	2	0	0	0	0	0	0	0	0	0	0	0			0			
3.conjunctiva	4	0	0	0	0	0	0	0	0	0	0	0						
iris	0	0	0	0	0	0	0	0	0	0	0	0			48h			
cornea	0	0	0	0	0	0	0	0	0	0	0	0	4	0	later is	0	(-)	(-)
Total score	4	0	0	0	0	0	0	0	0	0	0	0			0			
4.conjunctiva	2	0	0	0	0	0	0	0	0	0	0	0						
iris	0	0	0	0	0	0	0	0	0	0	0	0			48h			
cornea	0	0	0	0	0	0	0	0	0	0	0	0	2	0	later is	0	(-)	(-)
Total score	2	0	0	0	0	0	0	0	0	0	0	0			0			
Total score	10	0	0	0	0	0	0	0	0	0	0	0	10	0				
Average stimulus index	2.5	0	0	0	0	0	0	0	0	0	0	0	2.5	0	48h later is 0	0	(-)	(-)

Note: S and C stands for “sample” and “control”.

Summary: The sample is not irritant to the eye of rabbit.

Ⅳ、Guinea pig dermal allergy（sensitization）test

1. Materials and methods

1.1 Objective：the same as the objective described in the rat acute toxicity test .

1.2 test animals：Twenty Guinea pigs，provided by experimental animal center of Shanxi Medical University，body weight range was 200-250g.

1.3 test groups: Twenty Guinea pigs were divided randomly into two groups: test group and positive control group. Test group was stimulated with MOCA and positive control group was stimulated with 2，4-dinitrochlorobenzene.

1.4 Test steps:

a Sensitization:

Animals' left back were depilated $3\times3cm^2$ 24 hours before test. 0.1g MOCA was thoroughly mixed with sufficient quantum of distilled water and was applied on depilated field well-distributedly, covered with two layers bandages and one layer glass paper, fixed with non-stimulus adhesive tape. Wash it out with warm water after 6h. To repeat above-mentioned procedure on the 7th day and 14th day respectively. Positive control group was stimulated with 2，4-dinitrochlorobenzene,other procedure was the same as above.

b Provocation:

Animals' right back was depilated $2\times2cm^2$ 24 hours before test. On the 14th day after the last time of sensitization, 0.1g MOCA was thoroughly mixed with sufficient quantum of distilled water and was applied on right- depilated field well-distributedly, other procedure was the same as sensitization. Wash it out with warm water after 6h and keep the observation everyday for 12 days. Positive control group was challenged with 2，4-dinitrochlorobenzene,other procedure was the same as above.

2．Results：

Table 5 of sensitization test results

group	total number	positive number	rate of sensitization (%)
MOCA	10	0	0
2，4-dinitrochlorobenzene	10	10	100

Results: During test stage, animals in positive control group appeared erythema, dropsy and pachulosis to diversity extent; all of the animals in test group didn't appear erythema and edema. Rate of sensitization was 0, magnitude of sensitization was Ⅰ. So MOCA belong to weak-sensitizer.

V、Toxic symptoms and prevention measures:

Toxic symptoms: This chemical could be absorbed by undamaged skin. Kidney and liver can be damaged, but methemoglobinemia is not easy to be caused.

Prevention measures:

1. Skin: Put on poison-prevention-permeate working clothes and rubber gloves to make body safety. When contacting uncarefully, take off contaminant coat immediately and wash skin thoroughly with soapsuds or clear water.
2. Eyes: When contacting uncarefully, raise eyelid to wash with flowing clear water or normal sodium, and go to hospital to accept medical treatments.
3. Respiratory systems: Wear filter mask or air breathing respirator to prevent breathing uncarefully. If encountering emergency to evacuate, keep on apparatus respiratorius. If breathing uncarefully, break away from the spot to the place with fresh air, and keep respiratory tract unobstructed. When suffering dyspnea or respiration ceases, oxygen therapy and artificial respiration should be treated.
4. When eating by mistake, drink sufficient quantum warm of water or emetic, and go to hospital to receive medical treatments.
5. Other protections: Avoid smoking, eating and drinking at work spot. Exchange and wash working clothes in time. Don't drink wine before and after work. Shower with warm water. Take notice of detecting toxicant. Perform health examination regularly or before employment.

Explanations

1. The results of the report are only responsible for the submitted samples;

2. According to relevant laws , regulations and technical data, the results are reported only to submitted company or factory and administration management;

3. If you have any objections to the results, please propose within one month after you receive the report;

4. It is invalid if the report was changed or smeared or without the seal legally for toxicity identification.

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