APPENDICES

TOXICITY REPORT

ACUTE ORAL TOXICITY STUDY – TRZ-15 & TRZ-20

SUMMARY

Title: Acute oral toxicity study of TRZ-15 & TRZ-20 in mice

Test Compounds: TRZ-15 & TRZ-20

Species/Strain: Mice/Swiss albino

Gender: Female

No. of Test Animals: 09

Duration and Frequency of treatment: Single Dose

Route of Administration: Oral (diet-admixture)

Maximum Dose level: 2000 mg/kg

Volume of administration: -NA-

Vehicle: Pelleted chow

Post treatment examination period: 14 days

Type of examinations: Body weight
Clinical symptoms
Mortality
Gross necropsy

Results of the Study:

Administration of 2000 mg/kg TRZ15 & TRZ-20 showed no signs of toxicity or mortality during the test period

THE LD_{50} OF TRZ-15 & TRZ-20 IN MICE IS >2000 mg/kg

GENERAL INFORMATION

Type of Study:

The study was performed in accordance with the OECD gudielines (No. 423, 2001). Annex 2C of the guideline document was followed unless indicated otherwise. Accordingly, the initiation dose was 2000 mg/kg. Category 5 evaluation was precluded as a dose beyond 2000 mg/kg was unlikely to be ever used in practice.

Place of Study:

Shri G. H. Patel Pharmacy Building, Donors Plaza, Fatehgunj, Vadodara-390002 (A constituent of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda)

Study Sponsored by:

1. PhD Contingency of Mr. Anshuman Sinha, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda
2. UGC-BSR(RFMS)Contingency of Mr. Anshuman Sinha, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda

TEST SUBSTANCE INFORMATION

Name/Code: TRZ-15 & TRZ-20

Source: Synthesized and purified in the Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Reference: Compound Data-Sheet of TRZ-15 & TRZ-20 by Riyaz S. Tamboli, Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Appearance: Yellow powder

Storage: Room temperature, away from light

Safety requirement: Not Known

Expected Pharmacological Effect: Anticholinesterase and antioxidant property and could also be protective against $A\beta_{1-42}$ induced neurotoxicty.

Expiry Date: Not Known

Preparation: Required amount of TRZ-15 & TRZ-20 were weighed and suspended in 1 ml of 0.1% sodium carboxymethyl cellulose by triturition followed by vortexing immediately before administration.

ANIMALS USED FOR THE TEST

Species/Strain: Mice/Swiss Albino

Age at the commencement of test: 10-12 weeks

Body weight range: 25-30 g

Sex: Female

Total no. of Animals used: 09

Source of Animals: Zydus Research Centre, Ahmedabad, India

Acclimitization: 1 week

Randomization: 1 day prior to test compound administration

Justification for species and sex: Swiss albino mice are preferred for studies on compounds acting on the central nervous system eg. Scopolamine induced amnesic mice model. They present an appropriate mammalian system for replication of effects that might be observed upon administration of the test compounds to humans.

Husbandry: The animals were housed in polypropylene cages (19×42×28 cm^3) with paddy husk as bedding. Pelleted chow diet and drinking water were provided *ad libitum*. The room for the animals was maintained at 22°C ± 3°C with an RH of 40-70%. Temperature and humidity were recorded using a thermohygrometer.

DOSING

Groups:

Sr.No.	Substance	Dose# (mg/ kg)	Volume (ml/kg)	No. of Animals
1	Control group – 0.1%CMC	—	1	03
2	TRZ-15 suspended in 0.1%CMC	2000	1	03
3	TRZ-20 suspended in 0.1%CMC	2000	1	03

Dose Selection: Since no previous information about the in vivo data regarding the test compound was available, Annex 2C of the guideline was followed for dose selection. No pilot study or dose-ranging study was performed.

Mode of administration: orally
Justification for route of administration (ROA): This ROA is the intended ROA for further preclinical studies.
Dosing Frequency: Single dose
Applied maximum dose and volume: 2000 mg/kg
Dosing protocol: The animals were fasted overnight before dosing. The second day dosing was performed.

POST-TREATMENT EXAMINATION

The post-treatment examination period was 14 days from the date of dosing.
Body weight examination: Body weights of the animals were recorded on days 0, 7 and 14. Slight fluctuations were observed in the body weight of animals but since they were within 20% of the mean body weight no additional measurements were taken and any other precaution was not followed (Table1).

General behavior: The animals were closely observed during the first 6 hours after dosing. The animals were starved during this period with access to water. No other significant observations were recorded during this period. This part coincided with the light cycle and most of the time animals were asleep. Since the animals were provided with modified diets they initially showed a mild aversion towards diet consumption but later on consumed the diets at will. When awake, the animals showed normal grooming behavior and food (Table2) & water intake was also normal. No untoward observations were made until the terminal day of the study.

Mortality: Mortality was recorded twice daily but no mortality was found in any dose group until day 14.

Pathological Necropsy: At the end of the study period, the animals were euthanized and major organs (brain, heart, lung, liver, kidney, spleen) were harvested. Gross necropsy was performed by an individual blinded to the groups. No macroscopic lesions were recorded. Viscera, gastrointestinal tract and mucous linings appeared normal. Major blood vessels did not show any abnormalities.

Table1: Body Weight

Day 0	Body weight(in gms)*		
Animal Number	Control	2000 mg/kg (TRZ-15)	2000 mg/kg (TRZ-20)
1	30	29	30
2	29	30	28
3	28	26	27
Day 7	Body weight(in gms)*		
Animal Number	Control	2000 mg/kg (TRZ-15)	2000 mg/kg (TRZ-20)
1	30	30	29
2	29	30	28
3	29	27	28
Day 14	Body weight(in gms)*		
Animal Number	Control	2000 mg/kg (TRZ-15)	2000 mg/kg (TRZ-20)
1	31	31	30
2	30	29	29
3	29	28	28

*results are rounded off to nearest whole number

Table2: Daily food intake

Day No.	Total food intake/cage/3 animals (in gms)*		
	Control	2000 mg/kg (TRZ-15)	2000 mg/kg (TRZ-20)
1	0.6	0.5	0.6
2	0.6	0.5	0.5
3	0.5	0.4	0.4
4	0.4	0.5	0.4
5	0.4	0.6	0.5
6	0.6	0.4	0.6
7	0.4	0.6	0.4
8	0.4	0.5	0.5
9	0.4	0.5	0.4
10	0.6	0.5	0.6
11	0.5	0.4	0.5
12	0.5	0.5	0.5
13	0.4	0.6	0.5
14	0.5	0.5	0.6

*results are rounded off to nearest whole number

DEVIATIONS FROM THE GUIDELINE

No any deviations were attempted/perceived from the guideline for the toxicity study.

CONCLUSION

At the end of the study, no untoward observations were made regarding body weight, food intake or normal behavior. Gross necropsy did not reveal any suggestive lesions or abnormal anatomical feature. Hence it was concluded that the LD_{50} of TRZ-15 & TRZ-20 upon oral administration is >2000 mg/kg.

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End of Report

TOXICITY REPORT

ACUTE ORAL TOXICITY STUDY – 3b

SUMMARY

Title: Acute oral toxicity study of 3b in mice

Test Compound: 3b

Species/Strain: Mice/Swiss albino

Gender: Female

No. of Test Animals: 06

Duration and Frequency of treatment: Single Dose

Route of Administration: Oral (diet-admixture)

Maximum Dose level: 2000 mg/kg

Volume of administration: -NA-

Vehicle: Pelleted chow

Post treatment examination period: 14 days

Type of examinations: Body weight
Clinical symptoms
Mortality
Gross necropsy

Results of the Study: Administration of 2000 mg/kg 3b showed no signs of toxicity or mortality during the test period

THE LD_{50} OF 3b IN MICE IS >2000 mg/kg

GENERAL INFORMATION

Type of Study:

The study was performed in accordance with the OECD gudielines (No. 423, 2001). Annex 2C of the guideline document was followed unless indicated otherwise. Accordingly, the initiation dose was 2000 mg/kg. Category 5 evaluation was precluded as a dose beyond 2000 mg/kg was unlikely to be ever used in practice.

Place of Study:

Shri G. H. Patel Pharmacy Building, Donors Plaza, Fatehgunj, Vadodara-390002 (A constituent of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda)

Study Sponsored by:

1. PhD Contingency of Mr. Anshuman Sinha, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda
2. UGC-BSR(RFMS)Contingency of Mr. Anshuman Sinha, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda

TEST SUBSTANCE INFORMATION

Name/Code: 3b

Source: Synthesized and purified in the Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Reference: Compound Data-Sheet of 3b by Amit Verma, Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Appearance: Pale yellow powder

Storage: Room temperature, away from light

Safety requirement: Not Known

Expected Pharmacological Effect: Anticholinesterase and antioxidant property and could also be protective against $A\beta_{1-42}$ induced neurotoxicty.

Expiry Date: Not Known

Preparation: Required amount of 3b was weighed and suspended in 1 ml of 0.1% sodium carboxymethyl cellulose by triturition followed by vortexing immediately before administration.

ANIMALS USED FOR THE TEST

Species/Strain: Mice/Swiss Albino

Age at the commencement of test: 10-12 weeks

Body weight range: 25-30 g

Sex: Female

Total no. of Animals used: 06

Source of Animals: Zydus Research Centre, Ahmedabad, India

Acclimitization: 1 week

Randomization: 1 day prior to test compound administration

Justification for species and sex: Swiss albino mice are preferred for studies on compounds acting on the central nervous system eg. Scopolamine induced amnesic mice model. They present an appropriate mammalian system for replication of effects that might be observed upon administration of the test compounds to humans.

Husbandry: The animals were housed in polypropylene cages (19×42×28 cm^3) with paddy husk as bedding. Pelleted chow diet and drinking water were provided *ad libitum*. The room for the animals was maintained at 22°C ± 3°C with an RH of 40-70%. Temperature and humidity were recorded using a thermohygrometer.

DOSING

Groups:

Sr.NO.	Substance	Dose# (mg/ kg)	Volume (ml/kg)	No. of Animals
1	Control group – 0.1%CMC	—	1	03
2	3b suspended in 0.1%CMC	2000	1	03

Dose Selection: Since no previous information about the in vivo data regarding the test compound was available, Annex 2C of the guideline was followed for dose selection. No pilot study or dose-ranging study was performed.

Mode of administration: orally
Justification for route of administration (ROA): This ROA is the intended ROA for further preclinical studies.
Dosing Frequency: Single dose
Applied maximum dose and volume: 2000 mg/kg
Dosing protocol: The animals were fasted overnight before dosing. The second day dosing was performed.

POST-TREATMENT EXAMINATION

The post-treatment examination period was 14 days from the date of dosing.

Body weight examination: Body weights of the animals were recorded on days 0, 7 and 14. Slight fluctuations were observed in the body weight of animals but since they were within 20% of the mean body weight no additional measurements were taken and any other precaution was not followed (Table1).

General behavior: The animals were closely observed during the first 6 hours after dosing. The animals were starved during this period with access to water. No other significant observations were recorded during this period. This part coincided with the light cycle and most of the time animals were asleep. Since the animals were provided with modified diets they initially showed a mild aversion towards diet consumption but later on consumed the diets at will. When awake, the animals showed normal grooming behavior and food (Table2) & water intake was also normal. No untoward observations were made until the terminal day of the study.

Mortality: Mortality was recorded twice daily but no mortality was found in any dose group until day 14.

Pathological Necropsy: At the end of the study period, the animals were euthanized and major organs (brain, heart, lung, liver, kidney, spleen) were harvested. Gross necropsy was performed by an individual blinded to the groups. No macroscopic lesions were recorded. Viscera, gastrointestinal tract and mucous linings appeared normal. Major blood vessels did not show any abnormalities.

Table1: Body Weight

Day 0	Body weight(in gms)*	
Animal Number	Control	2000 mg/kg(3b)
1	25	25
2	27	26
3	28	29
Day 7	Body weight(in gms)*	
Animal Number	Control	2000 mg/kg(3b)
1	26	25
2	27	27
3	28	28
Day 14	Body weight(in gms)*	
Animal Number	Control	2000 mg/kg(3b)
1	27	26
2	27	28
3	29	28

*results are rounded off to nearest whole number

Table2: Daily food intake

Day Number	Total food intake/cage/3 animals (in gms)*	
	Control	2000 mg/kg (3b)
1	0.5	0.5
2	0.5	0.4
3	0.6	0.5
4	0.4	0.4
5	0.5	0.5
6	0.4	0.5
7	0.5	0.6
8	0.4	0.5
9	0.5	0.4
10	0.5	0.6
11	0.6	0.4
12	0.5	0.4
13	0.4	0.5
14	0.6	0.6

*results are rounded off to nearest whole number

DEVIATIONS FROM THE GUIDELINE

No any deviations were attempted/perceived from the guideline for the toxicity study.

CONCLUSION

At the end of the study, no untoward observations were made regarding body weight, food intake or normal behavior. Gross necropsy did not reveal any suggestive lesions or abnormal anatomical feature. Hence it was concluded that the LD_{50} of 3b upon oral administration is >2000 mg/kg.

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End of Report

TOXICITY REPORT

ACUTE ORAL TOXICITY STUDY – 19 & 28

SUMMARY

Title: Acute oral toxicity study of 19 & 28 in mice

Test Compound: 19 & 28

Species/Strain: Mice/Swiss albino

Gender: Female

No. of Test Animals: 09

Duration and Frequency of treatment: Single Dose

Route of Administration: Oral (diet-admixture)

Maximum Dose level: 2000 mg/kg

Volume of administration: -NA-

Vehicle: Pelleted chow

Post treatment examination period: 14 days

Type of examinations: Body weight
Clinical symptoms
Mortality
Gross necropsy

Results of the Study: Administration of 2000 mg/kg 19 & 28 showed no signs of toxicity or mortality during the test period

THE LD_{50} OF 19 & 28 IN MICE IS >2000 mg/kg

GENERAL INFORMATION

Type of Study:

The study was performed in accordance with the OECD gudielines (No. 423, 2001). Annex 2C of the guideline document was followed unless indicated otherwise. Accordingly, the initiation dose was 2000 mg/kg. Category 5 evaluation was precluded as a dose beyond 2000 mg/kg was unlikely to be ever used in practice.

Place of Study:

Shri G. H. Patel Pharmacy Building, Donors Plaza, Fatehgunj, Vadodara-390002 (A constituent of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda)

Study Sponsored by:

1. PhD Contingency of Mr. Anshuman Sinha, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda
2. UGC-BSR(RFMS)Contingency of Mr. Anshuman Sinha, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda

TEST SUBSTANCE INFORMATION

Name/Code: 19 & 28

Source: Synthesized and purified in the Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Reference: Compound Data-Sheet of 19 & 28 by Amit Verma, Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Appearance: Slight whitish liquid

Storage: Room temperature, away from light

Safety requirement: Not Known

Expected Pharmacological Effect: 5-HT_{2C} receptor agonists and could have therapeutic effects on rodent models of depression, anxiety, hypophagia and penile erection.

Expiry Date: Not Known

Preparation: Required amount of 19 & 28 were weighed and suspended in 1 ml of 0.1% sodium carboxymethyl cellulose by triturition followed by vortexing immediately before administration.

ANIMALS USED FOR THE TEST

Species/Strain: Mice/Swiss Albino

Age at the commencement of test: 10-12 weeks

Body weight range: 25-30 g

Sex: Female

Total no. of Animals used: 09

Source of Animals: Zydus Research Centre, Ahmedabad, India

Acclimitization: 1 week

Randomization: 1 day prior to test compound administration

Justification for species and sex: Swiss albino mice are preferred for studies on compounds acting on the central nervous system eg. Tail suspension test in mice. They present an appropriate mammalian system for replication of effects that might be observed upon administration of the test compounds to humans.

Husbandry: The animals were housed in polypropylene cages (19×42×28 cm^3) with paddy husk as bedding. Pelleted chow diet and drinking water were provided *ad libitum*. The room for the animals was maintained at 22°C ± 3°C with an RH of 40-70%. Temperature and humidity were recorded using a thermohygrometer.

DOSING

Groups:

Sr.NO.	Substance	Dose# (mg/ kg)	Volume (ml/kg)	No. of Animals
1	Control group – 0.1%CMC	—	1	03
2	19 suspended in 0.1%CMC	2000	1	03
3	28 suspended in 0.1%CMC	2000	1	03

Dose Selection: Since no previous information about the in vivo data regarding the test compound was available, Annex 2C of the guideline was followed for dose selection. No pilot study or dose-ranging study was performed.

Mode of administration: orally
Justification for route of administration (ROA): This ROA is the intended ROA for further preclinical studies.
Dosing Frequency: Single dose
Applied maximum dose and volume: 2000 mg/kg
Dosing protocol: The animals were fasted overnight before dosing. The second day dosing was performed.

POST-TREATMENT EXAMINATION

The post-treatment examination period was 14 days from the date of dosing.

Body weight examination: Body weights of the animals were recorded on days 0, 7 and 14. Slight fluctuations were observed in the body weight of animals but since they were within 20% of the mean body weight no additional measurements were taken and any other precaution was not followed (Table1).

General behavior: The animals were closely observed during the first 6 hours after dosing. The animals were starved during this period with access to water. No other significant observations were recorded during this period. This part coincided with the light cycle and most of the time animals were asleep. Since the animals were provided with modified diets they initially showed a mild aversion towards diet consumption but later on consumed the diets at will. When awake, the animals showed normal grooming behavior and food (Table2) & water intake was also normal. No untoward observations were made until the terminal day of the study.

Mortality: Mortality was recorded twice daily but no mortality was found in any dose group until day 14.

Pathological Necropsy: At the end of the study period, the animals were euthanized and major organs (brain, heart, lung, liver, kidney, spleen) were harvested. Gross necropsy was performed by an individual blinded to the groups. No macroscopic lesions were recorded. Viscera, gastrointestinal tract and mucous linings appeared normal. Major blood vessels did not show any abnormalities.

Table1: Body Weight

Day 0	Body weight(in gms)*		
Animal Number	Control	2000 mg/kg (19)	2000 mg/kg (28)
1	26	27	30
2	28	28	28
3	30	29	27
Day 7	Body weight(in gms)*		
Animal Number	Control	2000 mg/kg (19)	2000 mg/kg (28)
1	26	27	30
2	29	27	28
3	29	29	28
Day 14	Body weight(in gms)*		
Animal Number	Control	2000 mg/kg (19)	2000 mg/kg (28)
1	27	28	31
2	30	28	29
3	30	30	29

*results are rounded off to nearest whole number

Table2: Daily food intake

Day No.	Total food intake/cage/3 animals (in gms)*		
	Control	2000 mg/kg(19)	2000 mg/kg(28)
1	0.4	0.4	0.5
2	0.5	0.4	0.4
3	0.4	0.5	0.4
4	0.5	0.5	0.5
5	0.4	0.6	0.5
6	0.6	0.4	0.6
7	0.6	0.5	0.6
8	0.5	0.6	0.5
9	0.4	0.5	0.4
10	0.6	0.5	0.5
11	0.6	0.4	0.5
12	0.5	0.5	0.4
13	0.5	0.4	0.6
14	0.6	0.6	0.5

*results are rounded off to nearest whole number

DEVIATIONS FROM THE GUIDELINE

No any deviations were attempted/perceived from the guideline for the toxicity study.

CONCLUSION

At the end of the study, no untoward observations were made regarding body weight, food intake or normal behavior. Gross necropsy did not reveal any suggestive lesions or abnormal anatomical feature. Hence it was concluded that the LD_{50} of 19 & 28 upon oral administration is >2000 mg/kg.

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End of Report

TOXICITY REPORT

ACUTE ORAL TOXICITY STUDY – 3d

SUMMARY

Title: Acute oral toxicity study of 3d in mice

Test Compound: 3d

Species/Strain: Mice/Swiss albino

Gender: Female

No. of Test Animals: 06

Duration and Frequency of treatment: Single Dose

Route of Administration: Oral (diet-admixture)

Maximum Dose level: 2000 mg/kg

Volume of administration: -NA-

Vehicle: Pelleted chow

Post treatment examination period: 14 days

Type of examinations: Body weight
Clinical symptoms
Mortality
Gross necropsy

Results of the Study: Administration of 2000 mg/kg 3d showed no signs of toxicity or mortality during the test period

THE LD_{50} OF 3d IN MICE IS >2000 mg/kg

GENERAL INFORMATION

Type of Study:

The study was performed in accordance with the OECD gudielines (No. 423, 2001). Annex 2C of the guideline document was followed unless indicated otherwise. Accordingly, the initiation dose was 2000 mg/kg. Category 5 evaluation was precluded as a dose beyond 2000 mg/kg was unlikely to be ever used in practice.

Place of Study:

Shri G. H. Patel Pharmacy Building, Donors Plaza, Fatehgunj, Vadodara-390002 (A constituent of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda)

Study Sponsored by:

1. PhD Contingency of Mr. Anshuman Sinha, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda
2. UGC-BSR(RFMS)Contingency of Mr. Anshuman Sinha, PhD Student at Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda

TEST SUBSTANCE INFORMATION

Name/Code: 4

Source: Synthesized and purified in the Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Reference: Compound Data-Sheet of 3d by Amit Verma, Pharmaceutical Chemistry lab of Pharmacy Dept., Faculty of Tech. & Engg., The M. S. University of Baroda.

Appearance: Slight whitish liquid

Storage: Room temperature, away from light

Safety requirement: Not Known

Expected Pharmacological Effect: Selective dopamine D_1 receptor agonist and could be beneficial in rodent models of Parkinson's disease.

Expiry Date: Not Known

Preparation: Required amount of 3d was weighed and suspended in 1 ml of 0.1% sodium carboxymethyl cellulose by triturition followed by vortexing immediately before administration.

ANIMALS USED FOR THE TEST

Species/Strain: Mice/Swiss Albino

Age at the commencement of test: 10-12 weeks

Body weight range: 25-30 g

Sex: Female

Total no. of Animals used: 06

Source of Animals: Zydus Research Centre, Ahmedabad, India

Acclimitization: 1 week

Randomization: 1 day prior to test compound administration

Justification for species and sex: Swiss albino mice are preferred for studies on compounds acting on the central nervous system eg. MPTP induced Parkinson's mice model.

Husbandry: The animals were housed in polypropylene cages (19×42×28 cm^3) with paddy husk as bedding. Pelleted chow diet and drinking water were provided *ad libitum*. The room for the animals was maintained at 22°C ± 3°C with an RH of 40-70%. Temperature and humidity were recorded using a thermohygrometer.

DOSING

Groups:

Sr. NO.	Substance	Dose# (mg/ kg)	Volume (ml/kg)	No. of Animals
1	Control group – 0.1%CMC	—	1	03
2	3d suspended in 0.1%CMC	2000	1	03

Dose Selection: Since no previous information about the in vivo data regarding the test compound was available, Annex 2C of the guideline was followed for dose selection. No pilot study or dose-ranging study was performed.

Mode of administration: orally
Justification for route of administration (ROA): This ROA is the intended ROA for further preclinical studies.
Dosing Frequency: Single dose
Applied maximum dose and volume: 2000 mg/kg
Dosing protocol: The animals were fasted overnight before dosing. The second day dosing was performed.

POST-TREATMENT EXAMINATION

The post-treatment examination period was 14 days from the date of dosing.

Body weight examination: Body weights of the animals were recorded on days 0, 7 and 14. Slight fluctuations were observed in the body weight of animals but since they were within 20% of the mean body weight no additional measurements were taken and any other precaution was not followed (Table1).

General behavior: The animals were closely observed during the first 6 hours after dosing. The animals were starved during this period with access to water. No other significant observations were recorded during this period. This part coincided with the light cycle and most of the time animals were asleep. Since the animals were provided with modified diets they initially showed a mild aversion towards diet consumption but later on consumed the diets at will. When awake, the animals showed normal grooming behavior and food (Table2) & water intake was also normal. No untoward observations were made until the terminal day of the study.

Mortality: Mortality was recorded twice daily but no mortality was found in any dose group until day 14.

Pathological Necropsy: At the end of the study period, the animals were euthanized and major organs (brain, heart, lung, liver, kidney, spleen) were harvested. Gross necropsy was performed by an individual blinded to the groups. No macroscopic lesions were recorded. Viscera, gastrointestinal tract and mucous linings appeared normal. Major blood vessels did not show any abnormalities.

Table1: Body Weight

Day 0	Body weight(in gms)*	
Animal Number	Control	2000 mg/kg(3d)
1	30	27
2	26	30
3	28	27
Day 7	Body weight(in gms)*	
Animal Number	Control	2000 mg/kg(3d)
1	30	26
2	25	29
3	28	27
Day 14	Body weight(in gms)*	
Animal Number	Control	2000 mg/kg(3d)
1	31	27
2	26	30
3	29	28

*results are rounded off to nearest whole number

Table2: Daily food intake

Day Number	Total food intake/cage/3 animals (in gms)*	
	Control	2000 mg/kg(3d)
1	0.4	0.4
2	0.5	0.4
3	0.5	0.4
4	0.5	0.4
5	0.6	0.5
6	0.5	0.5
7	0.6	0.4
8	0.5	0.5
9	0.4	0.5
10	0.6	0.5
11	0.5	0.4
12	0.5	0.5
13	0.4	0.6
14	0.6	0.6

*results are rounded off to nearest whole number

DEVIATIONS FROM THE GUIDELINE

No any deviations were attempted/perceived from the guideline for the toxicity study.

CONCLUSION

At the end of the study, no untoward observations were made regarding body weight, food intake or normal behavior. Gross necropsy did not reveal any suggestive lesions or abnormal anatomical feature. Hence it was concluded that the LD_{50} of 3d upon oral administration is >2000 mg/kg.

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End of Report