International Journal of Pharmaceutical Archive-3(8), 2014, 486-492
Available online through www.ijpaonline.info ISSN 2319-7226

ACUTE AND SUB ACUTE TOXICITY STUDY ON SIDDHA DRUG ARUVAGAI CHOORANAM

RESEARCH ARTICLE

K Thangaraj*[1], Thanigaivel[2] and P Parthiban[1]

[1]Maruthuvam branch, government siddha medical college, chennai, (T.N.), India.
[2]Sairam advanced centre for research, chennai, (T.N.), India.

(Received on: 18-08-14; Revised & Accepted on: 26-08-14)

ABSTRACT

H*ypertension is the most important preventable risk factor for premature death worldwide. Aruvagai chooranam(AC) is a traditional siddha medicine for the treatment hypertension. To evaluate safety profile of AC in rats under the OECD guidelines 423 and 407. Sub-acute toxicity studies, different doses of AC were administered orally to rats once daily for 28 consecutive days in various dose levels ranging from 40, 200 and 400 mg/kg. In acute toxicity, study of AC revealed no mortality, abnormal signs and behavioral changes in rats at the dose of 2000 mg/kg body weight administered orally. Histopathological analysis did not reveal any abnormal macroscopic changes in the vital organs. The AC was found to be safe in animals. No toxic effect was observed in acute and sub acute toxicity study of AC.*

Keywords: *Aruvagai chooranam, Hypertension, Acute toxicity, Sub-acute toxicity.*

INTRODUCTION

Hypertension is the most important preventable risk factor for premature death worldwide[1].It increase the risk of ischemic heart disease[2]. Hypertension is more common in some ethnic groups, particularly back Americans and Japanese, and approximately 40-60% is explained by genetic factors[3]

Hypertension or high blood pressure, sometimes called arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated[4].

Hypertension is rarely accompanied by any symptoms, and its identification is usually through screening, or when seeking healthcare for an unrelated problem. A proportion of people with high blood pressure reported headache (particularly at occipital of the head and in the morning) as well as lightheadedness, vertigo, tinnitus,(buzzing or hissing in the ears),altered vision or fainting episodes[5].

The siddha drug Aruvagai Chooranam (AC) has been quoted by Brammamuni Vaithyasutharam-390 for the treatment of Hypertension[6].

The preclinical toxicity studies are essential for determining a safe dose for human trials Consequently an effort was made to evaluate acute and sub-acute toxicity of herbal siddha formulation of Aruvagai chooranam in laboratory animals.

MATERIALS & METHODS

Kothamalli(Coriandrum sativum) -60mg
Seeragam(Cuminum cyminum) -10mg
Karunseeragam(Nigella sativa -10mg
Sathakuppai(Anethum graveolens) -10mg
Elavangam(Syzygium aromaticum -10mg
Sirunagapoo(Mesua ferrea) -10mg
AthimathuramGlycyrrhiza glabra) -10mg
Seenakarkandu(Rack candy) -120mg

Corresponding author: K Thangaraj[1]*, E-mail: doctorgold24@gmail.com*

a) Raw drugs are purified and powdered.
b) Equal amount of senaakarkandu powder and equal amount of raw drugs powder are mixed well, and collected.

Animals: Rat of either sex weighing more than 150 gm were obtained from the animal house of King Institute of Preventive Medicine. The animals were used with the approval of the Institute animal ethics committee (IAEC) of Sairam Advanced Centre for Research, Chennai approval no. (1545/PO/a11/CPCSEA/1-3/2013). They were fed with a balanced standard pellet diet, maintained under standard laboratory condition providing 23±2°C temperature, standard light cycle (12 h light, 12 h dark) and water ad libitum.

The animals are randomly selected six animals per group, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Acute Toxicity Study – OECD 423 guidelines[7-8]:
Acute oral toxicity study for Aruvaai chooranam was carried out as per OECD Guidlines423.As with other sequential test designs, care was taken to ensure that animals are available in the appropriate size and age range for the entire study. The test substance is administered in a single oral dose by garage using a feeding needle. Animals were fasted prior to dosing. Following the period of Fasting, the animals were weighed and then test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours.

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days, to observe any death or changes in general behavior and other physiologically activities. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important especially if there is a tendency for toxic signs to be delayed. Observations include changes in skin and central nervous system, and somotomoter activity and behavior pattern. The animals were then observed daily for gross behavior chances and any other signs of acute toxicity [Table 1].

Sub-Acute Toxicity[9]:
In a 28-days, sub-acute toxicity study, was conducted under the OECD guidelines, forty either sex(5+5) rats were divided into four groups of 10 rats each. Group I that served as normal control was administered with distilled water (p.o) while group II, III and IV were administered daily with the Aruvagai chooranam (AC) for 28 days at a dose of 40, 200, 400 mg/kg respectively.

The weight of each rat was recorded on day 0 and weekly intervals throughout the course of the study [Table 2], food and water consumption per rat was calculated [Table3 & 4].

On 29th day, the animals were fasted for approximately 18 h, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

RESULT

Acute oral toxicity

Table 1: Dose finding experiment and its behavioral Signs of Toxicity

Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2000	+	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15.Lacrimation 16. Exophthalmia 17. Diarrhea 18. Writhing 19. Respiration 20. Mortality

Table 2: Body weight (g) changes of albino rats exposed to Aruvagai Chooranum for 28 days

Dose (mg/kg/day)	*Days*				
	1	**7**	**14**	**21**	**28**
Control	122.37±3.21	124.14±4.09	118.21±2.17	127.21±5.11*	133.32±1.89*
40	121.14 ±2.14	125.1 4 ±3.21	127.21±2.14	127.1 7 ±6.32	134.14 ±4.21*

200	125.22±6.27	127.31±6.24	130.12±1.23	131.17 ±4.20	135.32 ±5.62
400	126.12±5.23	128.54 ±1.21	130.2 4±1.61	131.07±7.26*	134.21±8.36*

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=6.

Table 3: Food (g/day) intake of albino rats exposed to Aruvagai Choranam for 28 days

Dose (mg/kg/day)	Days(g/rats)				
	1	**7**	**14**	**21**	**28**
Control	38.25±3.10	36.18±2.78	39.36±2.10	36.14±2.80	39.20±2.12
40	38.15±1.22	37.31 ±1.51	38.12 ±1.32	38.14 ±1.36	38.06 ±1.50
200	39.30 ±1.21	39.20 ±1.23	38.05 ±2.30	39.12±5.01	40.46±2.88
400	40.05 ±4.14	41.24±4.21	42.25 ±5.00	42.12±1.56	42.32±2.14

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=6.

Table 4: Water (ml/day) intake of albino rats exposed to Aruvagai Chooranam for 28 days

Dose	Days(ml/rat)				
(mg/kg/day)	**1**	**7**	**14**	**21**	**28**
Control	44.2468±3.00	43.32 ±3.22	45.21 ±3.14	43.32 ±2.54	42.10±2.96
40	47.32 ±5.21	47.12 ±6.12	48.12 ±3.12	49.45 ±3.21	50.1.70±3.20
200	48.1.75 ±1.25	48.30 ±1.36	47.42 ±2.55	48.45±2.23	48.27±1.27
400	49.15 ±2.63	48.30 ±3.25	48.48 ±1.02	49.31 ±3.41	50.24 ±2.12

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=6.

Hematological and blood biochemical analyses:

At the end of the study, the animals were fasted for approximately 18 h, then slightly anesthetized with ether on the 29th day. Blood samples for hematological and blood analyses were taken from retro-orbital plexus.

Heparinized blood samples were taken for determining complete blood count [**Table 5**] (white blood cell count, differential white blood cell count, platelet count, red blood cell count, hematocrit, and hemoglobin) by semi-automated hematology analyzer.

Table 5: Effect of Aruvagai Chooranam on Haematological parameters after 28 days

Parameter	Control	40 mg/kg	200 mg/kg	400 mg/kg
Redbloodcell (mm^3)	7.31±1.21	6.90±1.20	6.52±0.22	6.25±0.11
HB (%)	15.60 ±0.19	15.45 ±0.16	15.53 ±1.20	15.58 ±1.25
Leukocyte(x10^6/mL)	10100±100.51	10152±086.05	10201±046.11	10370±244.11
Platelets/ul	1325±39.32	1284±54.34	1295±52.27	1286±47.23
MCV (gl)	58.32 ±2.21	58.16 ±2.35	57.25 ±2.27	56.13 ±2.45
DLC (%)				
N	14.38 ±1.42	15.50 ±3.12	14.48 ±1.24	16.44 ±1.01
L	78.12±3.14	80.42±2.22	81.12±4.43	82.14±1.71
M	2.22 ±1.23	2.35 ±1.45	2.36 ±1.24	2.62 ±1.23
E	1.12±0.11	1.00±0.15	1.10±0.14	1.28±0.12
B	0	0	0	0
ESR(mm)	1±00	1±00	1±00	1±00

PCV	48.20±2.44	46.32±3.10	46.14±2.65	47.30±2.14
MCH pg	18.17 ±1.32	19.12 ±0.7 8	18.23 ±1.65	20.24 ±0.31
MCHC g/dl	30.04 ±1.35	31.31 ±2.63	32.69 ±0.85	33.18 ±1.23

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. N=6.

serum from non-heparinized blood was carefully collected for blood chemistry and enzyme analysis, glucose, Creatinine, total protein, albumin, total and direct bilirubins, serum glutamate-oxaloacetate trans-aminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) were automatically determined using auto analyzer [**Table 6,7,8&9**]

Table 6: Effect of Aruvagai Chooranam on Hepatic parameters

Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Total Bilirubin (mg/dL)	0.205±1.01	0.206±0.12	0.207±0.14	0.208±1.02
Bilirubin direct (mg/dL)	0.1±0.1 7	0.1±0.1 5	0.1±0.1 9	0.1±0.14
Bilirubin indirect(mg/dL)	0.1±00	0.1±00	0.1±00	0.1±00
SGOT (U/L)	166.44±3.24	164.78±2.52	162.42±3.45*	159.14±2.12*
SGPT(U/L)	45.4±2.14	44.4±2.28	44.00±2.15	45.62±4.13
Total Protein(g/dl)	10.62±1.30	10.16±0.30	9.42±0.27	10.11±0.46
Albumin(g/dl)	3.31 ±0.24	3.24 ±0.22	3.35 ±0.20	3.24 ±0.15
Globulin(g/dl)	6.12±0.24	5.24±0.30	6.77±0.34	6.48±0.10
A/G Ratio(g/dl)	0.55±0.21	0.54±0.21	0.65±0.41	0.63±0.5 0
GGT(U/L)	7.4±0	7.2±0	7.1±0	7.1±0

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. *vs. control group* N=6.

Table 7: Effect of Aruvagai Chooranam on Renal parameters

Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Urea(mg/dL)	64.24 ±3.11	63.26 ±1.25	64.12±5.17	68.44 ±2.14
Creatinine (mg/dL)	0.82±0.16	0.82±0.22	0.82±0.14	0.83±0.33
Uric acid (mg/dL)	1.60±1.20	1.59±1.23	1.57±1.30	1.56±1.02
Na m.mol	138.12±3.41	137.4±3.14	138.12±2.01	139.18±3.14
K m.mol	20. 24±2.11	19.10 ±4.24	20.280±3.62	20.13±5.58
Cl m.mol	99.50 ±2.34	100.51±2.18	98.18±2.28	102.24±2.20*

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. *vs. control group* N=6.

Table 8: Effect of Aruvagai Chooranam on Lipid profile

Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Total cholestrol(mg/dL)	41.24±1.35	43.22±2.12	40.12±1.02	44. 19±3.00
HDL(mg/dL)	12.40 ±1.45	12.27±1.20	12.29±1.36	13. 15±3.12
LDL(mg/dL)	38.18±2.88	37.14±2.47	42.11±3.18	35. 10±1.13
VLDL(mg/dl)	16.45 ±2.46	16.36 ±1.66	16.24 ±2.10	14. 14±1.88
Triglycerides (mg/dl)	82.14±1.23	81.11±1.110	81.22±1.35	83. 54±4.28
TC/HDL ratio (g/dl)	3.37±2.21	3.47±2.27	3.29±1.26	3. 21±1.36
Blood glucose(mg/dl)	110.16±8.62	112.37±4.12	112.0±3.33	112.20±2.28

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. *vs. control group* N=6.

Table 9: Effect of Aruvagai Chooranam on Urine parameters

Parameters	Control	40 mg/kg	200 mg/kg	400 mg/kg
Colour	Yellow	Yellow	Yellow	Yellow
Transparency	Clear	Cloudy	Slightly turbid	Turbid
Specific gravity	1.010	1.010	1.010	1.010
PH	>7.2	>8.0	>9.0	>8.0
Protein	Nil	2+	1+	2+
Glucose	Nil	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve	-ve
Ketones	-ve	+ve	+ve	+ve
Blood	Absent	Absent	Absent	Absent
Urobilinogen	Normal	Abnormal	Abnormal	Abnormal
Pus cells	0-cells/HPF	2-cells/HPF	1-cell/HPF	1-cell/HPF
RBCs	Nil	0-1cells/HPF	Nil	Nil
Epithelial cells	Nil	Nil	1-cell/HPF	1-cell/HPF
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01. *vs. control group* N=6.

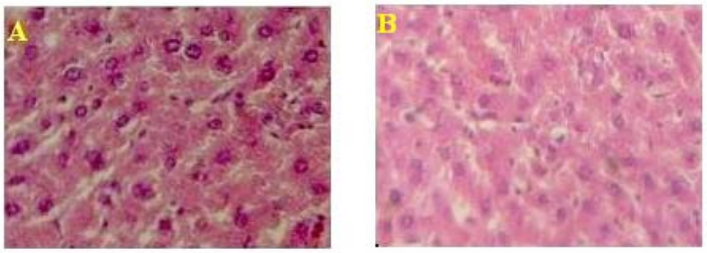
Necropsy: All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, adrenals, spleen, brain, heart, uterus and testes/ovaries were recorded [Table 10]. Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin and were examined microscopically [Panel-1, 2, 3, & 4]

Table 10: Effect of Aruvagai Chooranam on Organ weight

Dose (mg/kg)	Control	40 mg/kg	200 mg/kg	400 mg/kg
Liver (g)	5.24±0.14	4.75±0.20	4.23±0.22	4.45±0.21
Heart (g)	0.70±0.05	0.65±0.05	0.66±0.02	0.67±0.02
Lung (g)	1.78±0.25	1.45 ±0.22	1.74 ±0.10	1.53 ±0.21
Spleen (g)	0.74 ±0.07	0.69±0.05	0.66±0.04	0.62 ±0.05
Ovary (g)	1.91±0.14	1.67±0.18	1.66±0.15	1.86±0.12
Testes (g)	1.40±0.12	1.42±0.12	1.43±0.19	1.41±0.12
Brain (g)	1.43±0.18	1.44±0.16	1.44±0.18	1.42±0.17
Kidney (g)	0.70±0.05	0.72±0.05	0.72±0.04	0.71±0.04
Stomach (g)	1.23±0.10	1.30±0.17	1.23±0.12	1.12±0.20

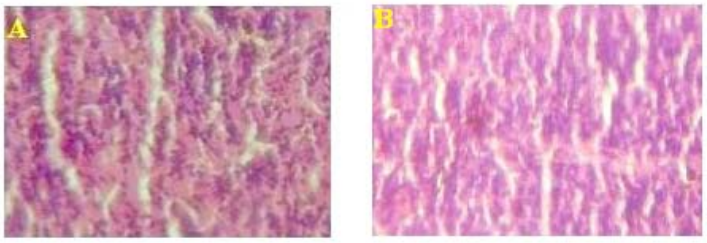
Values are mean of 6 animals ± S.D. (Dunnett's test). *P<0.05; **P<0.01 *vs control* N=6.

HISTOPATHOLOGY OF TOXICOLOGICAL STUDY



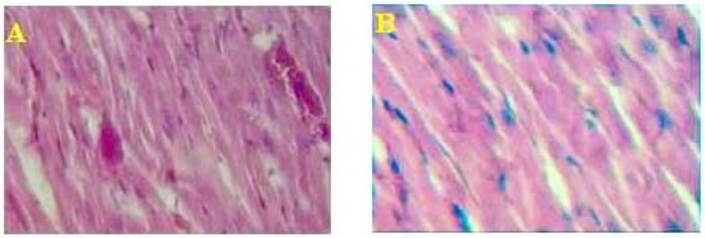
Panel 1: Light photomicrography of liver of a control rat

Figure A – Control
Figure B – Treated on high dose, no abnormality is seen in hepatocytes, sinusoids.



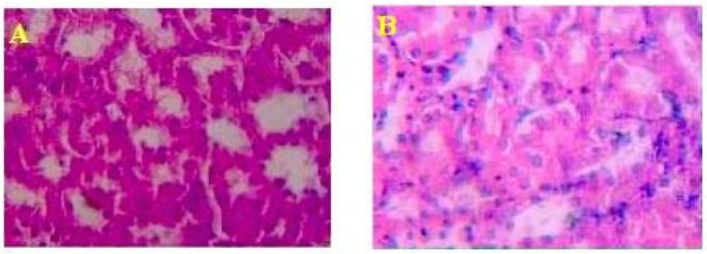
Panel 2: Light photomicrography of Spleen of a control rat

Figure A – Control
Figure B – Treated on high dose, no abnormality is seen in trabeculae, capsule.



Panel 3: Light photomicrography of Heart of a control rat

Figure A – Control
Figure B – Treated on high dose, no abnormality is seen in nuclei of Myocytes, myocardium



Panel 4: Light photomicrography of Kidney of a control rat

Figure A – Control
Figure B – Treated on high dose, no abnormality is seen in glomeruli, Bowman's capsule, capillaries.

Statistical analysis: Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way Anova followed by dunnet't' test using a computer software programme -INSTAT-V3 version.

DISCUSSION

The results of acute toxicity study of Aruvagai chooranam revealed no mortality, abnormal signs and behavioral changes in rats at the dose of 2000 mg/kg body weight administered orally. All animals from control and all the treated dose groups survived throughout the dosing period of 28 days. The results for body weight determination of animals from control and different dose groups show comparable body weight gain throughout the dosing period of 28 days. During dosing period, the quantity of food and water consumed by animals from different dose groups was found to be comparable and normal with that by control animals.

The results of hematological investigations such as Erythrocytes, Total Leucocytes and Platelets count conducted on day 29, revealed no significant changes in the values when compared with those of respective controls. This gave clear justification that Aruvagai chooranam did not influence bone marrow and spleen. Among the differential count of WBC, only the Eosinophil's count was slightly increased at the Aruvagai chooranam dosage of 40 mg/kg and 200 mg/kg. This might be occurred due to stress. Results of Biochemical investigations conducted on days 29, Urea, SGOT, SGPT, and Bilirubin were within the limits. LDL level was elevated in animals of 40 mg/kg dose group ($P<0.05$) and at the dosage of 400mg/kg, total cholesterol level was slightly increased but these were within the normal limits. The other cardio vascular risk markers were also within normal, ensured that Aruvagai chooranam did not influence the Cardio vascular system.

Urine analysis of control group and treated group of animals determined in week 4 did not reveal major abnormalities rather than transparency, pH and deposits. Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable with respective control group. Gross pathological examination of animals in control as well as the treated groups did not reveal any abnormalities. Histopathology: The vital organs such as liver, heart, Spleen and kidneys were removed from the test groups at the end of the study they did not reveal any abnormal macroscopic changes. Microscopically, these organs of the test groups revealed normal histological appearance when compared with the control group.

CONCLUSION

Based on these findings, no toxic effect was observed upto 2000 mg/kg of Aruvagai chooranam and 400 mg/kg of Aruvagai chooranam over a period of 28 days on oral route. So, it can be concluded that the Aruvagai chooranam can be prescribed for therapeutic use in human with the dosage recommendations of upto 400 mg/kg body weight p.o. our findings emphasise the need to implement effective and low cost management regimens, these findings result in limited conclusions More rigorously designed and powerd studies are needed.

REFERENCES

1. Global health risks: mortality and burden of disease attributable to selected major risks". World Health Organization. 2009. Retrieved 10 February 2012.
2. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R (December 2002). "Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies". Lancet **360** (9349): 1903–13.doi:10.1016/S0140-6736(02)11911-8. PMID 12493255.
3. Davidsons principle and practice of medicine -21st edition 2010,Churchill livingstone Elsever.
4. Carretero OA, Oparil S (January 2000). "Essential hypertension. Part I: definition and etiology". Circulation **101** (3): 329–35. doi:10.1161/01.CIR.101.3.329.PMID 10645931.
5. Marshall, IJ; Wolfe, CD; McKevitt, C (Jul 9, 2012). "Lay perspectives on hypertension and drug adherence: systematic review of qualitative research". BMJ (Clinical research ed.) **345**: e3953. doi:10.1136/ bmj.e3953. PMC 3392078.PMID 22777025.
6. Bhrammamuni vaithiasuthiram-390,page no-120-121.
7. Schlede E., Mischke U., Diener W. and Kayser D. The International Validation Study of the Acute-Toxic-Class Method (oral). Arch. Toxicol. 1994;69, 659-670.
8. Schlede E., Mischke U., Roll R. and Kayser D. A National Validation Study of the Acute-Toxic-Class Method – an alternative to the LD50 test. Arch. Toxicol. 1992; 66: 455-470.
9. OECD Guidelines for the Testing of Chemicals (No. 407, Section 4: Health Effects) "Repeated Dose 28-Day Oral Toxicity in Rodents" (Adopted on 12 May 198 1 and Updated on 27 July 1995).

Source of support: Nil, Conflict of interest: None Declared