Title : ACUTE ORAL TOXICITY TEST OF TRANSGENIC VEGETABLES IN RAT

Adoption: **OECD 401**

Application and limitation of tests

Acute oral toxicity is the adverse effects occuring within a short time of oral administration of a single dose of a test chemical or multiple doses given within 24 hrs. It is the initial step to find out median lethal dose (LD50) value which serve as basis for classification and labelling of the compound. It also forms a basis for selection of dose for subchronic studies. It will provide information on target organ toxicity after single exposure.

Principle

The test compound is administered orally by gavage in numerical doses to grous of animals, one dose per group. Signs of toxicity and death of animals are observed during 14 days observation period. The dead animals are necropsied during and the surviving animals are sacrificed and necropsied after the 14 days observation period for gross and histopathological studies, clinical biochemistry and haematological examiniation.

Description of the test Procedure

Animals

Healthy rats kept under standard animal husbandry conditions are used. At least 10 animals (male/female) are dosed. The weight variation of animals does not exceed 5-10g.

Animal maintenance

Animals are acclimatized to the experimental animal room having temperature 75 ± 2°F, humidity 30-70% and 12:12 hrs light dark condition. Animals are caged with maximum of 2 animlas in each polypropylene cages. Standard animal diet and water *ad libitum* is given to animals.

Preparation of dose

Test sample i.e. concentrated paste or cryogenic dehydrated powder of transgenic vegetables dissolved/suspended in groundnut oil is administrated to rat fasted overnight. The volume does not exceed 1 ml/100 g body weight. At least four doses of the test sample spaced in geometrical factor are selected. The treatment schedule of short term toxicity is given below:

Group 1	-	Control (normal diet)
Group 2	-	Non transgenic vegetables
Group 3	-	Transgenic vegetables

Limit test dose

If a test sample at 5000 mg/kg body weight produces no mortality, then other doses are not essential.

Observations

The dosed animals are observed twice daily for 14 days to record the signs of poisoning and death of animals. The signs of poisoning include tremor, convulsion, salivation, diarrhoea, lethargy, sleep, coma, dyspnea, nasal bleeding etc. The time of death of animals is recorded. The body weight, food and water intake is recorded daily and monitored weekly. All the animals (moribund/live) are sacrificed after 14 days and examined for gross and histopathological changes, clinical biochemistry and haematological examination.

Pathology

The liver, kidney, gonads, adrenals, spleen and brain are weighed immediately after autopsy. All animals are subjected for gross pathological changes. The vital organs like liver, kidney, brain, gonads, spleen, adrenal, thyroid, lungs, heart, stomach, duodnum, jejunum, colon, uterus, prostate are fixed in formal saline solution and tissues embeded in parafin wax and section cut at 6 um on rotary microtome. The prepared slides are then stained in haematoxylin eosin for microscopic examinations.

Haematology

Haematology is carried out in oxalated blood using standard methods of Wintrobe and Landsberg 1935 and Kolmer et. al. 1951. Blood is analysed for WBC, RBC, Hb differential leucocytes, clotting and prothrombin time and ESR.

Clinical Enzymes

Serum and blood are analysed for:

(i) Glutamic oxaloacetic transaminase (GOT), (ii) Glutamic pyruvic transaminase (GPT), (iii) Alakline phosphatase (Orthophosphoric monoester hydroxylase ALP), (iv) Bilirubin (v) Blood glucose (vi) Blood urea nitrogen, (BUN) (vii) Non Protein nitrogen, (NPN) by the method of Wootton (1982), (viii) Acetylcholinesterase (AchE) by the method of Hestrin 1949 and (ix) Protein by the method of Lowry et. al. 1951.

Calculations

LD50 values and its range are calculated by the procedure of Weil 1952 and toxicity rating is done by Gleasons et al. 1969. All observed data are recorded and calculated by appropriate methods. The statistical evaluation is done by Fisher's student t' test. The results are summerised in tabular form.

References

Weil, C.S., tables for convenient calculation of median effective dose (LD or ED) and instruction in their use. Biometrics, 8, 249, 1952.

Gleason, M.N., Gosselin, R.E., Hodge, H.C. and Smith, R.P. Clinical toxicology of commerical products. Acute posioning 3rd ed. Williams and williams, Baltimore, Maryland.

Report on Acute Oral Toxicity

Test Animal.....

Rats..... Sex : Male/Female.....

Test Sample
Solid, Liquid, any other

Nature of vehicle
dist. water, peanut oil, corn oil, any other

Date of experiment started .....

Date of experiment terminated .....

LD50.....mg/kg; Range..... to .....mg/kg

Dosage (mg/kg)	Animals Died/Dosed	Death	Symptoms of toxicity
1. Control			
2.			
3.			
4.			

Statistical Method

Gross Pathology

Observations

Conclusions

Toxicity Rating

Report on Acute Oral Toxicity

Test animal : Rat.

Test Chemical : Solid, Liquid, any other

Nature of vehicle: dist. water, peanut oil, corn oil, any other

Date of expt. started....., date of expt. terminated.....

FOOD (G) WATER (ML) INTAKE OF MALE OR FEMALE ANIMALS EXPOSED TO.....FOR 14 DAYS

Dosage (mg/kg/day)	**Days**													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
MALE														
1. Control														
2.														
3.														
4.														
FEMALE														
1.														
2.														
3.														
4.														

Report on Acute Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... date of expt. terminated : :.....

RELATIVE ORGAN WEIGHT OF MALE OR FEMALE ANIMALS EXOSED TO ..... FOR 14 DAYS

Dosage (mg/kg/day)	Liver	Kidney	Adrenalm	Heart	Spleen	Brain	Pituitary	Testes Ovary	Epididymis Uterus	Cervix	Vagina
MALE											
1. Control											
2.											
3.											
4.											
FEMALE											
1.											
2.											
3.											
4.											

*Organ weight / Body weight x 100

Report on Acute Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... date of expt. terminated :.....

BLOOD PICTURE OF MALE OR FEMALE ANIMALS EXPOSED TO:..... FOR 14 DAYS

Dosage (mg/kg/day)	RBC (x 10 mm)	WBC (x 10 mm)	Hb	PVC	platelet	Differential Leucocyte count (%)			
						Neutrophils	Lymphocytes	Monocytes	Eosinophils
MALE									
1. Control									
2.									
3.									
4.									
FEMALE									
1.									
2.									
3.									
4.									

Report on Acute Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... Date of expt. terminated:.....

BIOCHEMICAL CHANGES IN MALE OR FEMALE ANIMALS EXPOSED TO ..... FOR 14 DAYS

Dosage (mg/kg/day)	Blood Sugar	Alk. Phos.		Protein		GOT		GPT	
		Liver	Serum	Liver	Serum	Liver	Serum	Liver	Serum
MALE									
1. Control									
2.									
3.									
4.									
FEMALE									
1. Control									
2.									
3.									
4									

SUBCHRONIC (90 DAYS) ORAL TOXICITY TEST OF TRANSGENIC VEGETABLES IN RAT

Adoption: OECD 408

Application and limitation of test

Subchronic oral toxicity is the advsese effect occuring as a result of repeated daily oral dosing of a chemical to the animals. In the evaluation of toxic characteristics of a chemical the subchronic oral toxicity provides information on possible health hazards due to repeated exposure over a limited period of time. It will provide the information on target organ and the possibility of cumulation and for the selection of dose level for chronic studies.

Principle

The test compound is orally administered in three doses to animals for a period of 90 days. The animals are observed for any signs of toxicity and death during the period of exposure. Vital tissues of moribund and sacrificed animals are put for histopathological studies. Clinical biochemistry and haematological examinations are also made.

Description of the test Procedure

Rat is the preferred rodent model for subchronic oral toxicity studies. Healthy animals kept under standard animal husbandry conditions are used. At least 20 animals of 6-8 weeks old are used per group for three dose levels. The weight of the animals does not vary +20 g.

Animal maintenance

Animals are acclimatized to the experimental animal room having temperature (75+2 F), humidity (30-70%) and 12:12 hr light: dark conditions. Animals are given commercial feed and water *ad libitum*.

Preparation of dose

Test sample i.e. concentrated paste or cyyogenic dehydrated powder of transgenic vegetables dissolved/suspended in peanut oil is orally administered by gavage to animals consequantly (5 days/week) for 90 days. The selection of the dose is made on the basis of acute toxicity studies of the test chemical. At least three dose level, one maximum, one minimum and one intermediate are used. Consideration is given that the highest dose may result toxic effects without causing excessive lethality and lowest dose may not produce any toxic effects. A group of vechicle control is also used.

Limit test dose

If a test at one dose level of at least 1000 mg/kg/body weight (but expected human exposure may indicate the need for a higher dose level), using the procedures described for this study, produces no observable toxic effects, then a full study using three dose levels may not be considered necessary.

The treatment schedule is given below:

Group 1 - Control

Group 2 - Non transgenic vegetables

Group 3 - Transgenic vegetables

Observations

Animals are observed once daily to record the signs of posioning, like tremor, convulsion, diarrhoea, lethargy, dyspnea and nasal bleeding etc. The time of death is also recorded. The body weight, food and water intake is recorded daily and monitored weekly. At the end of 90 days animals are weighed and sacrificed.

Pathology

The liver, kidney, gonads, adrenals, spleen and brain are weighed immediately after autopsy. All animals are subjected for gross pathological changes. The vital organs like liver, kidney, brain, gonads, spleen, adrenal, thyroid, lungs, heart, stomach, duodenum, jejunum, colon, uterus, prostate are fixed in formal saline solution and tissues embeded in parafin wax and section cut at 6 um on rotary microtome. The prepared slides are then stained in haematoxylin eosin for microscopic examinations.

Haematology

Haematology is carried out in oxalated blood using standard methods of Wintrobe and Landsberg 1935 and Zolmer et. al. 1951. Blood is analysed for WBC, RBC, Hb differential leucocytes, clotting and prothrombin time and ESR. Immunoglobulin profile (IGM, IGA, IGE).

Clinical Enzymes

Serum and blood are analysed for

(i) Glutamic oxaloacetic transaminase (GOT), (ii) Glutamic pyruvic transaminase (GPT), (iii) Alakline phosphatase (Orthophosphoric monoester hydroxylase ALP), (iv) Bilirubin (v) Blood glucose (vi) Blood urea nitrogen, (BUN) (vii) Non protein nitrogen, (NPN) by the method of Wootton (1982), (viii) Acetylcholinesterase (AchE) by the method of Hestrin 1949 (ix) Protein by the method of Lowry et. al. 1951. (x) Serum histamine level.

Calculation and evaluation of data

All observed data are recorded and calculated by appropriate methods. The statistical evaluation is done by Fisher's student 't' test 1950. The results are summerised in tabular form.

References

Wintrobe, M. and Landsberg, J.W. A standard technique for blood sedimentation test. American J. Med. Sci. 189, 102, 1935.

Kolmer, K.A. Spaulding, E.H. and Robinson, H.W. Approved laboratory techniques Ved Scientifc Book Agency Calcutta, India, 1951.

Wootton, I.D.P. Microanalysis in Medical Biochemistry Sixth Edition, Churchill Ltd., London, 1982.

Hestrin, S.H. The reaction of Acetylcholine and other carboxylic acid derivatives with hydroxyl amine and its analytical applications J. Biol. Chem. 180, 249, 1949.

Lowry, O.H. Rosenburgh, N.J. Farr, A.L. and Randall, R.J. Protein measurement with the Folin Phenol reagent J. Biol. Chem. 193, 265, 1951.

Fisher, R.A. Statistical methods for research workers 11th edition Edinburgh Oliver and Boyd, 1950.

Report on Subchronic Oral Toxicity

Test Animal : Rat.

Test Chemical : Solid, Liquid, any other

Nature of vehicle : dist. water, peanut oil, corn oil, any other

Date of expt. started :..... , Date of expt. terminated :.....

FOOD (G) WATER (ML) INTAKE OF MALE OR FEMALE ANIMALS EXPOSED TO ..... FOR 13 WEEKS

Dosage (mg/kg/day)	Weeks												
	1	2	3	4	5	6	7	8	9	10	11	12	13
MALE													
1. Control													
2.													
3.													
4.													
FEMALE													
1.													
2.													
3.													
4.													

Report on Subchronic Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... date of expt. terminated : :.....

RELATIVE ORGAN WEIGHT OF MALE OR FEMALE ANIMALS EXOSED TO ..... FOR 13 WEEKS

Dosage (mg/kg/day)	Liver	Kidney	Adrenalm	Heart	Spleen	Brain	Pituitary	Testes Ovary	Epididymis Uterus	Cervix	Vagina
MALE											
1. Control											
2.											
3.											
4.											
FEMALE											
1.											
2.											
3.											
4.											

*$\frac{\text{Organ weight}}{\text{Body weight}} \times 100$

Report on Subchronic Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... date of expt. terminated :.....

BLOOD PICTURE OF MALE OR FEMALE ANIMALS EXPOSED TO:..... FOR 13 WEEKS

Dosage (mg/kg/day)	RBC (x 10 mm)	WBC (x 10 mm)	Hb	PVC	platelet	Differential Leucocyte count (%)			
						Neutrophils	Lymphocytes	Monocytes	Eosinophils
MALE									
1. Control									
2.									
3.									
4.									
FEMALE									
1.									
2.									
3.									
4.									

Report on Subchrpnic Oral Toxicity

Test Animal : Rat

Test Chemical : Solid, Liquid, any other

Nature of vehicle : Dist, water, peanut oil, corn oil, any other

Date of expt. started :..... Date of expt. terminated:.....

BIOCHEMICAL CHANGES IN MALE OR FEMALE ANIMALS EXPOSED TO ..... FOR 13 WEEKS

Dosage (mg/kg/day)	Blood Sugar	Alk. Phos.		Protein		GOT		GPT	
		Liver	Serum	Liver	Serum	Liver	Serum	Liver	Serum
MALE									
1. Control									
2.									
3.									
4.									
FEMALE									
1. Control									
2.									
3.									
4									

PRIMARY SKIN IRRITATION TEST OF TRANSGENIC VEGETABLES IN RABBIT

Adoption: OECD 404

Application and limitation of test:

The assessment and evaluation of the toxic characterestics of a substances, determination of the irritant effects on the skin of mammals is an important initial step. Information derived from the test serves to indicate the existence of possible hazard likly to arise from exposure of the skin to the test substance.

Principle

The substances to be tested is applied in a single dose to the skin of several experimental animals, each animal serving as its own control. The degree of irritation is read and scored at specified intervals and is further described to provide a complete evaluation of the effects. The duration of the study should be sufficient to evaluate fully the reversibility or irreversibility of the effects observed.

Description of the test procedure

Animals

At least three adult rabbit should be used. Additional animals may be required to clarify equivocal responses.

Animal maintenance

Animals are acclimatized to the experimental animal room having temperature 75+2F, humidity 30-70% and 12:12 hrs light dark cycle. Animals are caged with maximum of two animals in each cage. Standard animal diet and water *at libitum*.

Prepration of dose and limit test dose

Test sample i.e. transgenic vegetable at a dose of 0.5ml of liquid or 0.5g of solid is applied to the test side. The treatment schedule is given below:

Group 1 - Control

Group 2 - Non transgenic vegetables

Group 3 - Transgenic vegetables

Observations

Animals are observed for signs of erythema and oedema and the responses scored

at 30-60 minutes, and then at 24, 48, 72 hours and 7 and 14 days after patch removal. Dermal irriation is scored and recorded as per the grades given in the table below.

References

Draise, J.H. The Appraisal of Chemicals in Foods, Drugs, and Cosmetics pp, 46-48. Association of Food and Drug officials of united States, Austin, Texas 1959.

Draise, J.H. Appraisal of the Safety of chemicals in Foods, Drugs and Cosmetics; pp 46-59. Association of Food and Drugs official of the United States, Topeka, Kanasas 1965.

Evaluation of Skin Reaction

Erythema and Eschar Formation	***Value***
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Maximum possible - 4

Oedema Formation	
No Oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre) and extending beyond area of exposure)	4

Maximum Possible - 4

Title : IRRITATION TO MUCOUS MEMBRANE TEST OF TRANSGENIC SEED IN FEMALE RABBIT

Adoption : OECD 405

Application and Limitation of Test

In the assessment and evaluation of the toxic characteristics of a substance, determination of the irritant effects on the mucous membrane of the rabbit is an important step. Information derived from this study serves to indicate the existence of possible hazards likely to arise from exposure on the mucous membrane to the test substance.

Principle:

The substance tested is applied in a single dose to the mucous membrane of the experimental animals. Simultaneous animals in the control group are also taken. The degree of irrritation is read and scored at specific interval. The complete evaluation is then described. The duration of the study is sufficient to evaluate fully the dermal irritation.

Description of the test procedure

Health adult animals at least 3 in number are used in both experimental and control groups. Animals are kept in the experimental animal room having temperature (75 ± 2°F), humidity (30-70%), and 12:12 light: dark condition. Animals are fed conventional laboratory diet and water *ad libitum*.

A dose of 0.1 ml of liquid or 0.1 gm of solid or semisolid is applied to the upper vault of the vagina. Exposure duration is 4 hrs. Longer exposure may be indicated under certain conditions. At the end of the exposure period residual substance is removed where practicable using water or appropriate solvent without disturbing the epidermis. The treatment schedule is given below:

Group 1 - Control -
Group 2 - Non transgenic seed
Group 3 - Transgenic seed

Observation

Observation period is not fixed but is sufficient to evaluate fully the effects of the test substance. Normally it need not exceed 14 days after application. Animals are examined for signs of erythema and oedema and the responses scored at 30-60 minutes, 24, 48, 72 hrs and then at 7 and 14 days. Mucous membrane irritation is scored and recorded as per the grades given in table below:

References

Draise, J.H. The approaval of chemical in Food, Drug and cosmetics pp, 46-48. Association of Food and Drug Officials of United States, Austin Texas 1959.

Draise, J.H. Appraisal of the Safety of chemicals in Foods, Drugs and Cosmetics; pp 46-59. Association of Food and Drugs official of the United States, Topeka, kanasas 1965.

Evaluation of Skin Reaction

Erythema and Eschar Formation	***Value***
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Maximum possible - 4

Oedema Formation	
No Oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre) and extending beyond area of exposure)	4

Maximum Possible - 4