SUBCHRONIC (90 DAYS) ORAL TOXCITY OF LEAVES OF TRANSGENIC PLANTS IN MALE RABBIT

Adoption: **OECD guideline No. 408**

2. METHOD

A. Application and limitation of test

Subchronic oral toxicity

Subchronic oral toxicity is the adverse effects occuring as a result of the repeated daily oral dosing of a transgenic material/product to experimental animals for part (not exceeding 10 per cent) of the life span. In the assessment and evaluation of the toxic characteristics of a transgenic material/product, the determination of subchronic toxicity provides information on possible health hazards likely to arise from repeated exposures over a limited period of time. It will provide information in target organs, the possibilities of cumulation and can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure.

Dose is the amount of test substance administered. Dose is expressed as weight of test substance per unit weight of food (percent).

Principle of the test method

The transgenic and nontransgenic leaves are administered orally as part of the green vegetable diet (in addition to the standard rabbit pellet diet), one dose per group, for a period of 90 days. During the period of administration the animals are observed daily to detect sign of toxicity. Animals which die during the period of administration are necropsied and at the conclusion of the test all surviving animals are sacrificed and necropsied and histopathological examinations carried out. Clinical biochemistry and haematological examinations are also made.

B. Description of the Test Produre

Preparations

Healthy adult male rabbits are acclimatised to the laboratory conditions for atleast 5 days prior to the test. Before the test, animals are randomised and assigned to the treatment groups. The transgenic and nontransgenic leaves will be administered in the diet. All animals shall be dosed by the same method during the entire experimental period.

Species and number

Young adult male rabbits shall be used for the study. At the commencement of the study, the body weight variation of animals used should not exceed by ± 20 per cent of the mean weight. 10 animals per group shall be used.

Housing and feeding conditions

The temperature in the experimental animal room is maintained at 75+5°F (22 + 3° C) and the relative humidity at 30-70 per cent. Artificial lighting at a light:dark ratio of 12:12 is used. The animals are housed individually in standard rabbit cages.

For feeding, the leaves and conventional laboratory rabbit pallet diet are placed in separate enamel trays. Drinking water is provided ad libitum in another separate enamel bowl. Standard hygiene procedures will be implemented.

Animal model

Healthy adult male rabbits weighing 1.5-2 kg will be used for the study.

Sample administration

The transgenic and non-transgenic leaves will be administered as part of the diet. Leaves from both the transgenic and non-transgenic lines will be delivered each week. The fresh leaves are conserved in plastic bags in a refrigerator and the rabbits are fed fresh leaves every day. The leaves and the pellets are placed in separate enamel trays and the remainder collected after 24 hours, weighed and removed.

Dosage

The daily quantities of the leaves and pellets are fed for 90 days as per the following schedule:

Group	Leaves	Pellets
T1	No leaves	Ad libitum
T2	10% TL	Ad libitum
T3	ad libitum Tl	Ad libitum
T4	10% NL	Ad libitum
T5	Ad libitum NL	Ad libitum

TL: *Transgenic leaves*
NL: *Non-transgenic leves*

If pair feeding (PF) is required, two more groups i.e. one PF TL and one PF NL will be added.

Laboratory Investigations

Animals will be observed daily for the changes in skin and fur, detail untoward

CNS, respiratory, ocular and gastrointestinal symptoms, for haematuria from day 1 through day 90. The consumption of diet (leaves and pellet), water as well as body weight and feed efficiency ofthe animals will be recorded daily.

The death, if any, will also recorded daily in the morning and the dead animal is examined for pathological changes. At the end of 90 days, all animals are weighed and sacrificed.

Clinial examination

The following examination are made at the end of 90 days of exposure:

(a) ***Haematology***: Haematocrit, haemoglobin concentration erythrocyte count, total and differential leucocyte counts, ESR, and a measure of clotting potential i.e. clotting time, prothrombin time and immunoglobulin profile are evaluated.

(b) ***Biochemistry:*** Clinial diagnostic enzymes such as liver and serum GOT, GPT, alkaline phosphatase and LDH are assayed. The levels of protein, glucose, serum bilirubin, blood urea nitrogen, non protein nitrogen and serum histamine are also evaluated.

(c) ***Pthology*** : *Gross necropsy:* All animals will be subjected to a full gross necropsy which included examination of the external surface of body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. Microscopic examination will be conducted by closely observing the various organs, viz. stomach, jejunum, ileum, colon, spleen, pancreas, heart, brain, liver, kidney, adrenals, thymus, thyroid, prostate and testes. The above organs will be weighed wet as soon as possible after dissection to avoid drying.

Histopathology

Full histopathology will be carried out on above organs of all animals in the control and dosed groups. All gross lesions will be examined.

The tissues are fixed in formalin, embedded in paraffin wax, sectioned at 6-8 microns and stained with Haematoxilin-Eosin for microscopic examinations

Data and Reporting

All observed results, quantitative and incidental, will be evaluated by appropriate, geneally accepted statistical methods. The data are summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions the types of lesions and the percentage of animals displaying each type of lesions.

Evaluation of results

The findings of the subchronic oral toxicity study should be considered in terms of the toxic effects and the necropsy and histopathological findings. The evaluation will

include the presence or absence, the incidence and severity, of abnormalities, including behavioural and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic, effects.

Test report

- The test report will include the following informations:
- species/strain used;
- toxic response date by sex and dose;
- time of death during the study or whether animals survived to termination;
- toxic or other effects;
- the time of observation of each abnormal sign and its subsequent course;
- food and body weight data;
- ophtholmological effects;
- haematological tests employed and results with relevant baseline data;
- clinical biochemistry tests employed and results with relevant baseline data;
- necropsy findings;
- detailed description of all histopathological findings; and
- statistical treatment of results where appropriate.

Report on Subchronic Oral Toxicity

Test Animal : Rabbit (Male)

Test Chemical : Leaves, administered is part of diet.

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 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.													

Report on Subchronic Oral Toxicity

Test Animal : Rabbit

Test Chemical :Leaves administered as part of diet.

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

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

RELATIVE ORGAN WEIGHT OF MALE ANIMALS EXPOSED TO ..... FOR 13 WEEKS

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

*Organ weight / Body weight x 100

Report on Subchronic Oral Toxicity

Test Animal : Rabbit, Male

Test Chemical : Leaves administered as part of diet.

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

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

Blood Picture of Male 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.									

Report on Subchronic Oral Toxicity

Test Animal : Rabbit, Male

Test Chemical : Leaves administered as part of diet.

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

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

BIOCHEMICAL CHANGES IN MALE 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.									