
STUDY TITLE

Report

Genetically Modified Potato Meal
Subchronic toxicity study in Wistar rats
Administration in the diet for 3 months**PERFORMING LABORATORY**Experimental Toxicology and Ecology
BASF Aktiengesellschaft
67056 Ludwigshafen, Germany**LABORATORY PROJECT IDENTIFICATION**

Project No.: 50C0066/04012

**VOLUME III OF III
(SUPPLEMENT)**

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TRANSLATION

LUFA-ITL

LUFA-ITL Gutenbergstr. 75-77, 24116 Kiel
BASF AKTIENGESELLSCHAFT
GV/TD -Z470/ Dr. Mellert

67056 LUDWIGSHAFEN

Date March 25, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 279892

Order no.: 155732
Date of receipt March 18, 2004
Name of sample: sample 2: test substance; freezedried potatoes
04/0066-1
Packaging Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	64.2		OS	VDLUFA III 7.2.1
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Date March 25, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 279893

Order no.: 155732
Date of receipt: March 18, 2004
Name of sample: sample 3: test substance; freezedried potatoes
04/0067-1
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	71.4		OS	VDLUFA III 7.2.1
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Date April 28, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 293235

Order no.: 161734
Date of receipt April 22, 2004
Name of sample: sample 1: test substance; freezedried potatoes mother
variety prevalent
Test substance no.: 04/0066-1
Packaging Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	75.3		OS	VDLUFA III 7.2.1
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Date April 28, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 293236

Order no.: 161734
Date of receipt April 22, 2004
Name of sample: sample 2: vehicle, KLIBA maintenance diet
Packaging Plastic box

Parameter of analyses method	unit	result	declaration	substance
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Nutritive value/contents

starch	%	35.2	OS	VDLUFA III 7.2.1
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Date April 28, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 293237

Order no.: 161734
Date of receipt: April 22, 2004
Name of sample: sample 3: preparation; 50,000 ppm, freezedried potatoes
mother variety prevalent
Test substance no.: 04/0066-1 (homogeneity: top)
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	37.1		OS	VDLUFA III 7.2.1
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Date April 28, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 293238

Order no.: 161734
Date of receipt: April 22, 2004
Name of sample: sample 4: preparation; 50,000 ppm, freezedried potatoes
mother variety prevalent
Test substance no.: 04/0066-1 (homogeneity: mid)
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	38.1		OS	VDLUFA III 7.2.1
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Date April 28, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 293239

Order no.: 161734
Date of receipt April 22, 2004
Name of sample: sample 5: preparation; 50,000 ppm, freezedried potatoes
mother variety prevalent
Test substance no.: 04/0066-1 (homogeneity: bottom)
Packaging Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	38.6		OS	VDLUFA III 7.2.1
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Date April 28, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 293240

Order no.: 161734
Date of receipt April 22, 2004
Name of sample: sample 6: test substance; freezedried potatoes EH92-527-1
Test substance no.: 04/0067-1
Packaging Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	70.9		OS	VDLUFA III 7.2.1
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Date April 28, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 293241

Order no.: 161734
Date of receipt April 22, 2004
Name of sample: sample 7: preparation; 50,000 ppm, freezedried potatoes
EH92-527-1
Test substance no.: 04/0067-1 (homogeneity: top)
Packaging Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	37.7		OS	VDLUFA III 7.2.1
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Date April 28, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 293242

Order no.: 161734
Date of receipt April 22, 2004
Name of sample: sample 8: preparation; 50,000 ppm, freezedried potatoes
EH92-527-1
Test substance no.: 04/0067-1 (homogeneity: mid)
Packaging Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	38.0		OS	VDLUFA III 7.2.1
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Date April 28, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 293243

Order no.: 161734
Date of receipt April 22, 2004
Name of sample: sample 9: preparation; 50,000 ppm, freezedried potatoes
EH92-527-1
Test substance no.: 04/0067-1 (homogeneity: bottom)
Packaging Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

Parameter of analyses	unit	result	declaration	substance	method
starch	%	37.9		OS	VDLUFA III 7.2.1

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Date June 30, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 325379

Order no.: 177866
Date of receipt: June 25, 2004
Name of sample: sample 1: test substance; freeze-dried potatoes mother variety prevalent
Test substance no.: 04/0066-1
Identification of the product: Date of food mixture: May 18, 2004
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

Parameter of analyses	unit	result	declaration	substance	method
starch	%	74.2		OS	VDLUFA III 7.2.1

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Date June 30, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 325380

Order no.: 177866
Date of receipt June 25, 2004
Name of sample: sample 2: vehicle, KLIBA maintenance diet
Identification of the product: Date of food mixture: May 18, 2004
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	37.0		OS	VDLUFA III 7.2.1
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Date June 30, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 325381

Order no.: 177866
Date of receipt: June 25, 2004
Name of sample: sample 3: preparation; 50,000 ppm, freezedried potatoes
mother variety prevalent
Test substance no.: 04/0066-1 (homogeneity: top)

Identification of the product: Date of food mixture: May 18, 2004
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	38.4		OS	VDLUFA III 7.2.1
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Date June 30, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 325382

Order no.: 177866
Date of receipt: June 25, 2004
Name of sample: sample 4: preparation; 50,000 ppm, freeze-dried potatoes
mother variety prevalent
Test substance no.: 04/0066-1 (homogeneity: mid)

Identification of the product: Date of food mixture: May 18, 2004
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	38.7		OS	VDLUF-III 7.2.1
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Date: June 30, 2004
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TEST RESULT

Analysis no.: 325383

Order no.: 177866
Date of receipt: June 25, 2004
Name of sample: sample 5: preparation; 50,000 ppm, freeze-dried potatoes
mother variety prevalent
Test substance no.: 04/0066-1 (homogeneity: bottom)

Identification of the product: Date of food mixture: May 18, 2004
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	39.0		OS	VDLUFA III 7.2.1
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TEST RESULT

Analysis no.: 325384

Order no.: 177866
Date of receipt: June 25, 2004
Name of sample: sample 6: test substance; freezedried potatoes EH92-527-1
Test substance no.: 04/0067-1
Identification of the product: Date of food mixture: May 18, 2004
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	70.4		OS	VDLUFA III 7.2.1
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Date June 30, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 325385

Order no.: 177866
Date of receipt: June 25, 2004
Name of sample: sample 7: preparation; 50,000 ppm, freeze-dried potatoes
EH92-527-1
Test substance no.: 04/0067-1 (homogeneity: top)

Identification of the product: Date of food mixture: May 18, 2004
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	38.6		OS	VDLUFA III 7.2.1
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Date June 30, 2004
Customer no.: 10027166
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TEST RESULT

Analysis no.: 325386

Order no.: 177866
Date of receipt: June 25, 2004
Name of sample: sample 8: preparation; 50,000 ppm, freeze-dried potatoes
EH92-527-1
Test substance no.: 04/0067-1 (homogeneity: mid)

Identification of the product: Date of food mixture: May 18, 2004
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	38.7			OS	VDLUFA III 7.2.1
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Date June 30, 2004
Customer no.: 10027166
page 1 of 1

TEST RESULT

Analysis no.: 325387

Order no.: 177866
Date of receipt: June 25, 2004
Name of sample: sample 9: preparation; 50,000 ppm, freezedried potatoes
EH92-527-1
Test substance no.: 04/0067-1 (homogeneity: bottom)

Identification of the product: Date of food mixture: May 18, 2004
Packaging: Plastic box

Parameter of analyses unit result declaration substance method

Nutritive value/contents

starch	%	38.4		OS	VDLUFA III 7.2.1
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**Functional Observational Battery (FOB):
Detailed description of examinations, ranking and documentation procedures**

The following describes in detail the scope of examinations and the ranking of the findings.

HOME CAGE OBSERVATIONS:**Posture**

The posture of the animals is examined for any abnormal findings.

Ranking:

- 0 animal is sitting or lying
- 1 animal is staying and moving
- 2 squatting posture
- 3 abdominal position
- 4 abdominal position with splayed limbs
- 5 lateral position
- 6 oblique head posture
- 7 opisthotonus

Tremors

Any tremors are documented.

Ranking:

- 0 no tremors
- 1 slight tremors
- 2 moderate tremors
- 3 severe tremors

Convulsions

If convulsions are seen, the severeness is assessed. If possible, also the type of convulsions, i.e. clonic (alternate muscular contraction and relaxation in rapid succession) or tonic (characterized by continuous tension) is documented.

Ranking:

- 0 no convulsions
- 1 slight convulsions
- 2 moderate convulsions
- 3 severe convulsions

Abnormal movements

Any abnormal movement or bizarre behaviour is documented.

Ranking:

- 0 no abnormalities
- 1 manege movements
- 2 head shaking
- 3 excessive cleaning
- 4 frequent chewing

Impairment of gait

Any abnormal movement or bizarre behavior is documented.

Ranking:

- 0 animal is not walking during observation
- 1 no impairment of gait
- 2 stiff gait
- 3 slight impairment of coordination, unsteady gait
- 4 moderate impairment of coordination, shuffling gait
- 5 severe impairment of coordination, dragging of the hindlimbs
- 6 severe impairment of coordination, with splayed limbs
- 7 animal is unable to walk (abdominal or lateral position)

Other findings*Ranking:*

- 0 no other findings
- * If findings appear which are not included in this grading system, they are described

OPEN FIELD OBSERVATIONS**Behavior when removed from cage**

The animal is removed from the home cage, and the reaction is documented.

Ranking:

- 0 animal is tense, but it shows no resistance against handling
- 1 animal shows a slight resistance against the handling
- 2 animal shows no resistance against the handling but appears indifferent
- 3 animal is difficult to handle, it shows aggressiveness
- 4 animal is very difficult to handle, it shows severe aggressiveness

Fur

The fur of the animals is examined for any abnormal signs.

Ranking:

- 0 nothing abnormal detected
- 1 discolored fur
- 2 urine staining of anogenital region
- 3 Piloerection
- 4 Alopecia
- 5 reduced care on fur

Skin

The visible parts of the skin (e.g. nose) are examined for any abnormal signs.

Ranking:

- 0 nothing abnormal detected
- 1 discolored skin
- 2 reddening
- 3 paleness
- 4 Dehydration (exsiccosis)
- 5 Hypothermia (skin is cold during handling)
- 6 lesion(s)
- 7 crust(s)

Salivation

The area around the mouth is examined for signs of discharge; if possible, also the color of discharge is documented.

Ranking:

- 0 no salivation
- 1 slight salivation (area around the mouth is moist)
- 2 moderate salivation (wet mouth)
- 3 severe salivation (mouth very wet, wet paws)

Nose discharge

Any discharge is documented.

Ranking

- 0 no discharge, dry nose
- 1 clear discharge
- 2 reddish discharge

Lacrimation

Any lacrimation or secretion of pigmented tears is documented. If possible, also the color of the tears is assessed.

Ranking:

- 0 no lacrimation
- 1 slight lacrimation
- 2 moderate lacrimation
- 3 severe lacrimation

Eyes/Pupils size*Ranking:*

- 0 Nothing abnormal detected, pupils contracted at room light
- 1 Chromodacryorrhoe
- 2 Exophthalmus
- 3 Pupils dilated
- 4 Abnormal shape of pupils
- 5 Oblique eye posture
- 6 Opacity
- 7 Cataract

Posture

The posture of the animals is examined for any abnormal findings.

Ranking:

- 0 animal is sitting or lying
- 1 animal is staying and moving
- 2 squatting posture
- 3 abdominal position
- 4 Abdominal position with splayed limbs
- 5 lateral position
- 6 oblique head posture
- 7 Opisthotonus

Palpebral closure

The level of eyelid closure is examined. Usually, the eyelids of rats are completely opened (except winking).

Ranking:

- 0 nothing abnormal detected
- 1 eyelid(s) slight closure
- 2 eyelid(s) half closure
- 3 eyelid(s) permanent closure

Respiration

The respiration is examined for any abnormalities.

Ranking:

- 0 nothing abnormal detected
- 1 respiration labored
- 2 gasping/respiratory sounds
- 3 respiration accelerated
- 4 respiration irregular

Tremors

Any tremors are documented.

Ranking:

- 0 no tremors
- 1 slight tremors
- 2 moderate tremors
- 3 severe tremors

Convulsions

If convulsions are seen, the severeness is assessed. If possible, also the type of convulsions, i.e. clonic (alternate muscular contraction and relaxation in rapid succession) or tonic (characterized by continuous tension) is documented.

Ranking:

- 0 no convulsions
- 1 slight convulsions
- 2 moderate convulsions
- 3 severe convulsions

Abnormal movements/stereotypy

Any abnormal movements or bizarre behavior are documented

Ranking:

- 0 no abnormalities
- 1 manege movements
- 2 head shaking
- 3 excessive cleaning
- 4 frequent chewing

Impairment of gait

If any impairment of gait is observed, the type and severeness is documented. Different types of gait impairment are ataxia (irregularity of muscular action), paralysis (loss of motor function) and paresis (slight or incomplete paralysis). If possible, also the affected limbs (forelimbs, hindlimbs) are documented.

Ranking:

- 0 animal is not walking during observation
- 1 no impairment of gait
- 2 stiff gait
- 3 slight impairment of coordination, unsteady gait
- 4 moderate impairment of coordination, shuffling gait
- 5 severe impairment of coordination, dragging of the hindlimbs
- 6 severe impairment of coordination, with splayed limbs
- 7 animal is unable to walk (abdominal or lateral position)

Activity/Arousal level

The activity of the animals in the open field and the arousal level is examined for any deviation from normal.

Ranking:

- 0 Normal exploration of the area
- 1 reduced exploration of the area
- 2 Severe reduced exploration of the area, animal apathetic
- 3 Increased exploration of the area, sudden or jerky movements
- 4 Hyperactivity

Feces excreted during the observation period

The appearance and consistency of the feces are described. The number of scybala is documented separately.

Ranking:

- 0 no defecation during observation period
- 1 Feces without abnormalities
- 2 discolored feces
- 3 crumbly feces
- 4 soft feces
- 5 muicid feces
- 6 diarrhea

Urine excreted during the observation period

The amount and color of the urine is described.

Ranking:

- 0 no urination during observation period
- 1 urine without abnormalities (some wet areas on the filter paper)
- 2 discoloration of urine
- 3 polyuria (great wet areas on the filter paper)

Number of rearings

In the open field, the animals usually rear several times in order to explore the new environment. A rear is counted, if both forelimbs are removed from the floor. The number of rearings is counted within a 2 minute observation period.

Ranking:

- (number)

SENSORY-MOTORIC TEST/REFLEXES

Approach response

A stick is approached slowly to the head of the animal. Normal reaction of the animal is to approach to the object or to ignore the object.

Ranking:

- 0 no reaction
- 1 approaching to object
- 2 escape reaction
- 3 aggressive reaction and attacking of object

Touch Response

The animal is touched with a stick along the flank. Normal reaction is to orientate to the stimulus or to ignore the stimulus.

Ranking:

- 0 no reaction
- 1 orientation to the stimulus
- 2 escape after touch
- 3 aggressive reaction and attacking of object
- 4 reaction to the stimulus but no ability to localize (e.g. turning to wrong side)

Vision ("visual placing response")

The animal is held on the tail and moved slowly towards the wire cover of a cage. Normal reaction of the animal is to stretch the forelimbs in order to grasp the object.

Ranking:

- 0 nothing abnormal detected (grasping with forelimbs)
- 1 no grasping

Pupillary reflex

The capacity of the pupils to adapt to darkness and to light is examined. The animals are put into a dark box for about 10 seconds and the pupils are examined using an electric torch. Normally, the pupil dilates in the dark and contracts immediately in the light.

Ranking:

- 0 nothing abnormal detected, physiological adaptation of the pupil to light
- 1 retarded adaptation of the pupil to light
- 2 no adaptation of the pupil to light, pupils permanently contracted
- 3 no adaptation of the pupil to light, pupils permanently dilated

Pinna reflex

The pinna of the animal is touched with a small bristle. Normally, the animals jerks the pinna or head immediately after the stimulus.

Ranking:

- 0 immediate response to the stimulus
- 1 no response to the stimulus

Audition ("startle response")

The animal is exposed to a sharp, electrically produced noise and the reaction to the sound is tested. Normally the animal shows a startle response (e.g. moving the ears, jerking, jumping backwards).

Ranking:

- 0 nothing abnormal detected, immediate normal response to the stimulus
- 1 no response
- 2 increased response
- 3 hyperreaction

Coordination of movements ("righting response")

The animal is turned into dorsal position and released. Normal reaction is that the animal immediately turns to an upright position.

Ranking:

- 0 nothing abnormal detected, immediate righting response
- 1 retarded righting response
- 2 fails to turn into upright position, animal stays in lateral position
- 3 no righting response, animal stays in dorsal position

Behavior during handling*Ranking:*

- 0 normal behavior, easy to handle, animal is tense, but it shows no resistance against handling
- 1 very easy to handle, animal is limply hanging in the hand
- 2 slightly difficult to handle, animal shows a slight resistance against handling
- 3 difficult to handle, animal shows a severe resistance against handling

Vocalization*Ranking:*

- 0 No or only sporadic vocalizations when touched
- 1 very frequent vocalizations when touched
- 2 vocalizations always when touched
- 3 vocalization without touching

Pain perception ("tail pinch")

The tip of the tail is squeezed with a forceps. Normally the animals responds to the stimulus, e.g. it moves away.

Ranking:

- 0 nothing abnormal detected, immediate response to the stimulus
- 1 weak or retarded reaction to the stimulus
- 2 no response to the stimulus
- 3 hyperreaction to the stimulus

Other findings*Ranking:*

- 0 no other findings
- * If findings appear which are not included in this grading system, they are described

Grip strength of forelimbs and hindlimbs

Measurement of grip strength is performed according to the method described by Meyer et al. 1979 (Neurobehavioral Toxicology, 1, 233-236):

The animal is brought into a position that forelimbs/hindlimbs are able to grip the metal bar of a newtonmeter. Then the animal is pulled gently backwards until it releases the grid. The average of two measurements is taken for each hindlimbs and forelimbs.

Documentation:

- (number)

Landing foot splay test

The animal is held about 30 cm above and dropped onto a flat area covered with putty. This results in clearly visible footprints. The distance between the fourth digits of the left and right hindlimb is measured. The average of two measurements is taken for each rat.

Documentation:

- (number)

GENERAL REMARK:

Details of localization or color, or detailed descriptions of findings (e.g. tonic or clonic convulsions) are be described using an abbreviation (letter or a combination of letters), if they are not given already in the SOP.