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ACUTE INHALATION TOXICITY OF NORASYSTEM®

Study number: PS07B164

Sponsor:

Name	PHODE
Address	Z.I. Albipôle 81150 ALBI TERSSAC
Telephone	+33 (0) 5 63 77 80 60
Fax	+33 (0) 5 63 77 80 61

Testing facility:

Name	PHYSIOSTIM
Address	Zone industrielle de Brénas 81440 Lautrec, France
Telephone	33 (0)5 63 70 81 07
Fax	33 (0)5 63 70 81 08

Study schedule:

Starting date of experimental work:	27/03/2007
End date of experimental work:	30/03/2007
Date of submission of the report:	20/04/2007

REPORT APPROVAL

PhysioStim

Testing Facility Management / Deputy Study Director

Marie LE GRAND Date

Study Director

Laetitia MERCADIEU Date

Sponsor

Sandrine GARRIGUES Date

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FINAL STUDY REPORT APPROVAL

Study Director

I, Laetitia MERCADIEU, Study Director, hereby confirm that the study PS07B164 was performed according to PhysioStim's standard operating procedures and to the final study plan PS07B164.PF with a validated method.

The final study report fully and accurately reflects the raw data generated during the study. There were no influences, impacts or circumstances noted which might have impaired the integrity of the study.

	Name	Date	Signature
Study Director	L. Mercadieu		

Scientific Management

I, the undersigned, certify that I reviewed this report on study PS07B164 and I concurred with its contents.

	Name	Date	Signature
Testing Facility Management	M. Le Grand		

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TABLE OF CONTENTS

1 - INTRODUCTION

The purpose of the study was to investigate the possible toxic effects of a 4-hours inhalation of test article "NORASYSTEM®" at the highest dose level, and to compare them to those obtained in a control group (6 animals of either sex in each group).

In the assessment and evaluation of the toxic characteristics of an inhalable material, such as a gas, volatile substance or aerosol/particulate, determination of acute inhalation toxicity was a necessary step. Acute inhalation toxicity was the total adverse effects caused by a substance following a single uninterrupted exposure by inhalation over a short period of time to a substance capable of being inhaled.

It provided information on health hazards likely to arise from short-term exposure by the inhalation route.

2 - RESULTS

According to the study plan PS07B164.PF, rats were weighed at their reception, on the day of experiment (J) and at one and two days later (J+1, J+2).
An analysis of the evolution of the rats' weight was done in order to reveal effects of the test article on this parameter.

2.1 - Weight gain

Two statistical tests were perfomed:

- ✓ An intragroup analysis to study the weight gain in a same group during the different phases of the experiment.
- ✓ An intergroup analysis to compare the weight gain between two different groups.

2.1.1 - *Intragroup analysis*

The average weight of each animal group on the day of experiment and at one and two days later is presented in the Figure 1. Individual weights are presented in the Appendix 2.

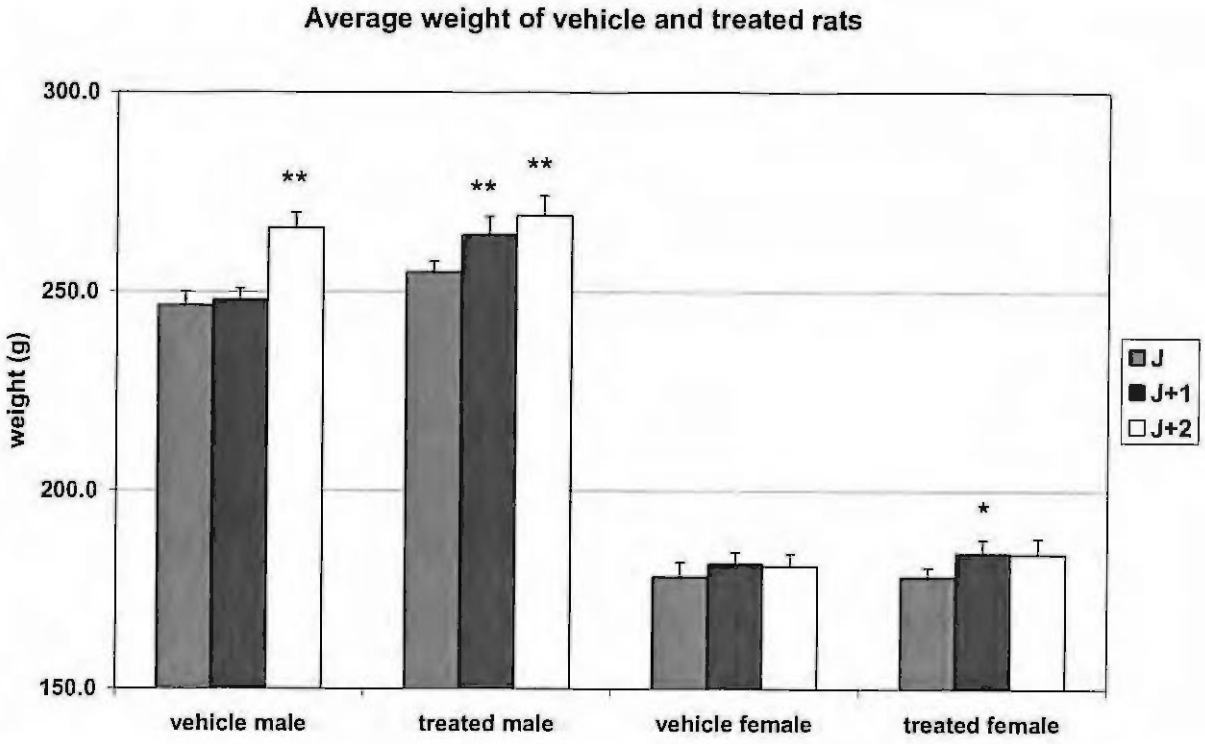

Fig. 1: Evolution of the rats' weight during the experimentation.

** $p<0.05$ and ** $p<0.01$ (Dunnett's test performed if ANOVA was significant).*

The weight gain in the male vehicle group was significant at J+2 compared to the experimental day (266.0 ± 3.8 g vs 246.7 ± 3.3 g; $p<0.01$).

In the same way, treated males significantly grew at J+1 (264.3 ± 4.6 g vs 255.0 ± 2.7 g; $p<0.01$) and at J+2 (269.0 ± 5.1 g vs 255.0 ± 2.7 g; $p<0.01$).

On the contrary, no significant growth was observed in the vehicle female group from J to J+2.

A slight but significant weight gain was observed at J+1 in the female treated group compared to the experimental days (184.3 ± 3.4 g vs 178.3 ± 2.3 g; $p<0.05$).

2.1.2 - Intergroup analysis

The comparison of the average weight between groups on the day of experiment (J) is presented in the Figure 2. Average weight gain at J+1 and J+2, expressed as absolutes changes from the experimental day are presented in the Figure 3. Individual weights are presented in the Appendix 2.

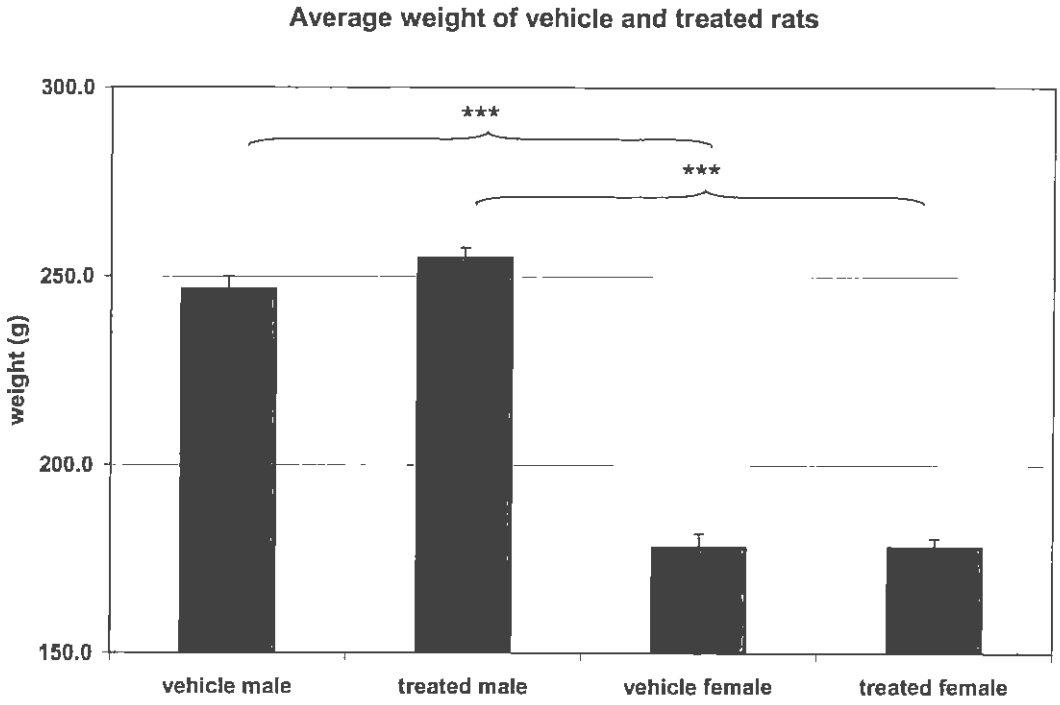

Fig. 2: Rats' weight at the experimentation day (J).

*** *p<0.001 (Unpaired Student t-test).*

The difference of weight between male and female was normal because all the rats were 7 weeks old at their reception, and a male rat is always, by definition, bigger than a female.

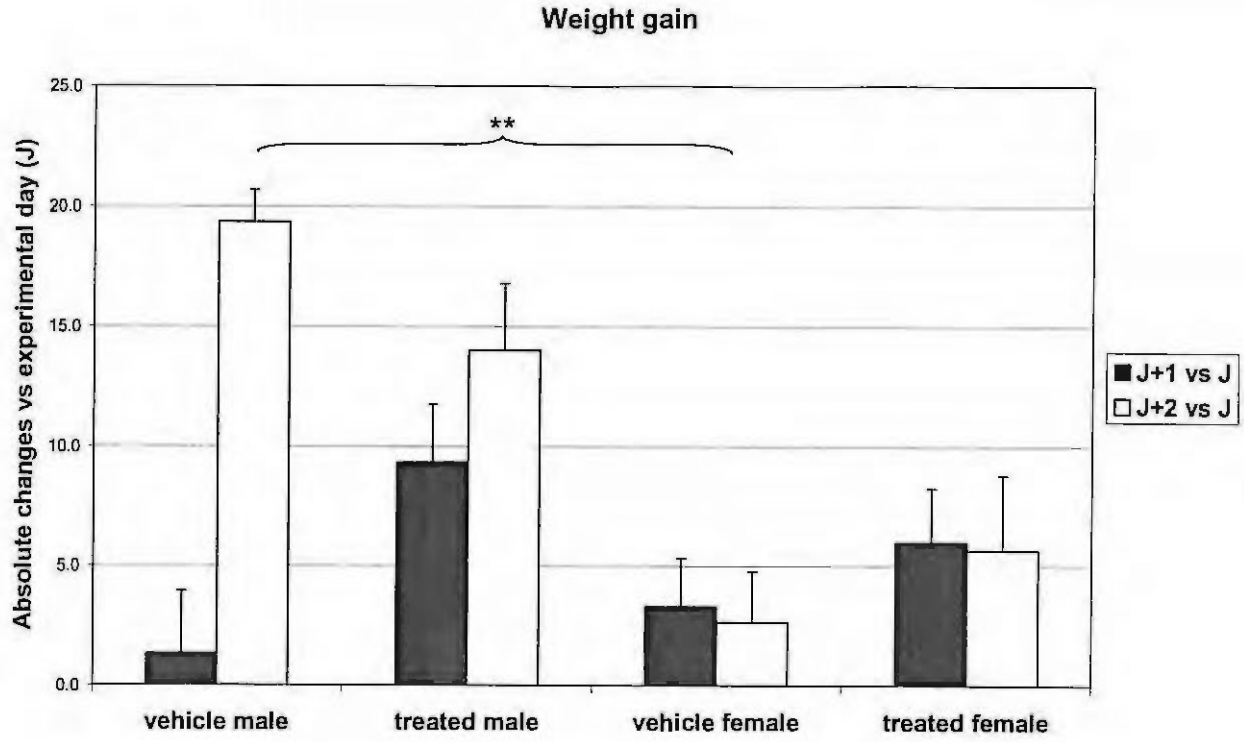

Fig. 3: Weight gain for each group at J+1 and J+2.

**** p<0.05 (Bonferroni's test performed if ANOVA was significant).*

No significant differences of weight were observed between vehicle and treated males and between vehicle and treated females (Figure 2).

Moreover, as shown in the Figure 3, the weight gain was not statistically different between vehicle and treated males and between vehicle and treated females on the two days following the experimental day.

A significant difference of weight gain was however observed between vehicle males and vehicle females (+19.3 ± 1.3 g vs +2.7 ± 2.1 g respectively; $p<0.01$; Figure 3).

2.2 - Behavioural analysis

The experimentation phase was recorded (DVD files) according an organization of the cage and a specific sharing out of the rats (Appendix 3).

The general and abnormal behaviour of the rats was observed and listed in the following tables.

2.2.1 - Male vehicle group

∑ : all the rats

Time	Rat	description
10'	∑	Exploration
15'	cage 3	Rest, side of the entry of air
25'	cage 1	Brawls, games, moving
	cage 2 & 3	Rest, side of the entry of air
31'	cage 1: n°4	One rat dug the litter, but not blocked the entry of air
50'	cage 1: n°1	Always active
	∑	Receptive to the stimuli
1h	cage 2	Rest at the entry of the air except for cage 2
1h30	cage 1	Awake, side of the entry
	cage 2	Awake, opposite side of the entry
1h48	cage 1: n°4	**The breathing movements were easily visible**
1h50	cage 2	Active
2h	cage 2 & 3	Active
2h10	cage 1 & 2	Rest
2h25	∑	Receptive to the stimuli
2h30	cage 1: n°4	Awake, draw up
2h42	cage 2	Opposite side of the entry of air
3h00	cage 1	One rat was awake, others were very calm
3h40	cage 3	Only one was awake
until 4h	∑	Calm, in rest

There was no death during the experimentation.

No abnormal behaviour was observed; animals explored the cage during a first phase, and in a second phase, tended to sleep since the rest phase of rats occurs normally during the light circadian period.

However, we could note that this group tended to sleep near the entry of the air.

2.2.2 - Male treated group

Time	Rat	Description
5'	∑	Exploration of the cage
7'50	∑	Rats were receptive to the experimenter
9'00		Calm
9'23	cage 1	**The breathing movements were easily visible**
10'35 to 12'00	cage 1: n° 8	**ONE RAT BLOCKED THE ENTRY OF THE VENTILATION WITH THE LITTER**
12'45	cage 1	Experimenter withdrew the litter at the entry
16'58	cage 1: n° 8	Tried to bury itself into the litter
17'20	cage 1: n° 8	**ONE RAT BLOCKED THE ENTRY OF THE VENTILATION WITH THE LITTER**
26'50	cage 1	Experimenter withdrew the litter at the entry
34'	cage 1	Rest, opposite side of the entry
	cage 3	one rat was awake
36'30	cage 1	strange position (conscious)
38'30		**The breathing movements were easily visible**
39'30	cage 1	Receptive to the diffusion of the test article
1h05	cage 2	Awake
1h23		Rest: rats were in the opposite side of the entry of product
1h48	cage 3	Awake
2h19'15	cage 1	**The breathing movements were easily visible**
2h27		Receptive: answer to the stimuli, cage 3 more slowly
2h43		Rest, grooming: rats were in the opposite side of the entry of product
3h00	cage 1	Awake
3h25	cage 2	Awake
3h35	cage 2	Awake and present violent behaviour
3h40 until 4h	∑	Rest: rats were in the opposite side of the entry of product

Compared to the vehicle group, we could observe some difference:

- ✓ They often checked a stand up position.
- ✓ We could observe that rats sniffed the air during the perfusion of the test article and then tried to block it by digging the litter.

2.2.3 - Female vehicle group

Time	Rat	description
5'	Σ	Brawls, games, moving
8'	Σ	Grooming, calm
6'50	cage 3	One rat was already in a phase of rest
10'	cage 2	Exploration around the entry of the air
15'	cage 1	Rest, side of the entry of air
	cage 2	Exploration
	Σ	Receptive to the outside stimuli
22'	cage 1	Observation of all the corners of the cage
25'	cage 2	Rats around the entry of the air
35'	cage 1	Active around the entry of air
	cage 2	Rest, opposite side of the entry of air
	cage 3	Rest, side of the entry of air
57'	**cage 2**	**N° 2 dug the litter, like it tried to block the entry**
58'	**cage 1**	**The breathing movements were easily visible**
until 1h	Σ	Active phase
1h	Σ	Rats in rest, receptive to the outside stimuli
2h25	cage 3	Brawls, games, moving
2h50	**cage 2**	**One rat seemed to try to block the entry of the air by digging the litter**
2h57	cage 2	N°5 dug the litter
3h30	cage 1	One rat ate the litter
until 4h	Σ	No abnormal behaviour

There was no death during the experimentation.

No abnormal behaviour was observed; animals explored the cage during a first phase, and in a second phase, tended to sleep since the rest phase of rats occurs normally during the light circadian period.

However, we could note that this group tended to sleep near the entry of the air.

A digging behaviour was observed near the entry of the air but this was not as clear as that observed in the treated groups.

2.2.4 - Female treated group

Time	Rat	description
	Σ	Exploration of the cage
8'	cage1	Exploration of the cage
	cage 2	Brawls
	cage 3	Rest
27'	cage 1: 6 & 8	Active rats
	Σ	Rats were in the opposite side of the entry of air
29'	Σ	Rest
45'	cage 3	Sniffed the entry of air, often drawn up
48'	8 & 10	Active, grooming, drawn up
1h04	cage 2	Tried to bury itself into the litter
	cage 1: 8	Rats were drawn up and present olfactory behaviour
	cage 2	Rats were drawn up and present olfactory behaviour
1h05	cage 2	Games, mouvements
1h05'15	cage 1:8	CHARACTERISTIC OLFACTORY BEHAVIOUR
1h06	cage 1:8	Rat was drawn up
1h06'50	cage 1	Games, movements
1h07	cage 2	CHARACTERISTIC OLFACTORY BEHAVIOUR
1h11'20	cage 1	Rats were scraping, licking
1h15	cage 2 & 3	Calm: receptive to the stimuli
1h20	Σ	Rats were in the opposite side of the entry of air
1h45	cage 1	**The breathing movements were easily visible**
1h47'25	cage 3	**ONE RAT BLOCKED THE ENTRY OF THE VENTILATION WITH THE LITTER**
1h54	Σ	Rest: rats were in the opposite side of the entry of air
2h04	cage 2	Games, movements, brawls
2h17	Σ	Rest: rats were in the opposite side of the entry of air
3h05	cage 2	**ONE RAT BLOCKED THE ENTRY OF THE VENTILATION WITH THE LITTER**
3h11	cage 1	Active
3h28	cage 1	Active
At the end of the video, the mechanism of perfusion was recorded		

Compared to the vehicle group, we could observe some difference:

- ✓ They often checked a stand up position.

- ✓ We could observe that rats sniffed the air during the perfusion of the test article and then tried to block it by digging the litter.

3 - CLINICAL DATA

The observations during and after the experimental phase for all groups are described below:

- General behaviour
- Changes in skin
- Changes in fur
- Changes in mucovis membranes
- Convulsions
- Tremors
- Salivation
- Lethargy
- Coma
- Hair
- Diarrhoea
- Drowsiness
- Nasal discharge
- Ocular discharge
- Changes in respiratory
- Wink eyes

None abnormal signs and none symptoms were revealed.

- No mortality was observed throughout the study

4 - CONCLUSION

The male rats were heavier than females and the weight gain in the vehicle male group was more important than that observed in the vehicle female group.

The body weight gain of the animals were not affected by treatment with the test product - NORASYSTEM® - as well for males than females.

Moreover, NORASYSTEM® did not affect animals behaviour except a tendency to block the perfusion system by digging the litter.

Finally, the limit test performed – that is to say the test at the highest exposure concentration for 4 hours – produced no compound-related mortality.

5 - APPENDICES

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Appendix 1: Study plan

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ACUTE INHALATION TOXICITY OF NORASYSTEM®

Study number: PS07B164

Sponsor:

Name	PHODE
Address	Z.I. Albipôle 81150 ALBI TERSSAC
Telephone	+33 (0) 5 63 77 80 60
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Testing facility:

Name	PHYSIOSTIM
Address	Zone industrielle de Brénas 81440 Lautrec, France
Telephone	33 (0)5 63 70 81 07
Fax	33 (0)5 63 70 81 08

Study schedule:

Estimated starting date of experimental work:	26/03/2007
Estimated end date of experimental work:	06/04/2007
Estimated date of submission of the report:	20/04/2007

STUDY PLAN APPROVAL

PhysioStim

Testing Facility Management

Marie LE GRAND — Date 23/03/07

Study Director

Laetitia MERCADIEU — Date 23/03/07

Deputy Study Director

Marie LE GRAND — Date 23/03/2007

Sponsor

Sandrine GARRIGUES — Date 28 mars 2007.

Doc. reference : PS07B164.PF

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ORGANISATION OF THE STUDY

SPONSOR

Sponsor contact:	Sandrine GARRIGUES Tel: +33 (0)5 63 77 80 60 Fax: +33 (0)5 63 77 80 61 E-mail: sgarrigues@phode.fr

TESTING FACILITY

Testing Facility Management:	M. LE GRAND Direct Tel: +33 (0)5 63 70 89 92 E-mail: marie.legrand@physiostim.com
Study Director:	L. MERCADIEU Direct Tel: +33 (0)5 63 70 89 96 E-mail: laetitia.mercadieu@physiostim.com
Deputy Study Director:	M. LE GRAND Direct Tel: +33 (0)5 63 70 89 92 E-mail: marie.legrand@physiostim.com
Quality Assurance:	M. CAPARROS Direct Tel: +33 (0)5 63 70 89 93 E-mail: myriam.caparros@physiostim.com

Communication between those concerned will be by phone, by fax or by e-mail.

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LIST OF ABBREVIATIONS

%: percent
°C: Celsius degree
CE: Communauté Européenne
g: gram
GLP: Good Laboratory Practices
h: hour
kg: kilogram
L: Liter
mg: milligram
n: number of experiment
OECD: Organization of Economics Cooperation and Development

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1 - INTRODUCTION

1.1 - Objectives and rationale of the study

The purpose of the study is to investigate the possible toxic effects of a 4-hours inhalation of test article "NORASYSTEM®" and to compare them to those obtained in a control group (6 animals of either sex in each group).
In the assessment and evaluation of the toxic characteristics of an inhalable material, such as a gas, volatile substance or aerosol/particulate, determination of acute inhalation toxicity is an initial test. Acute inhalation toxicity is the total adverse effects caused by a substance following a single uninterrupted exposure by inhalation over a short period of time to a substance capable of being inhaled.
It provides information on health hazards likely to arise from short-term exposure by the inhalation route.
The protocol used follows the spirit of the OECD 403 Guideline for testing of chemicals (12 May 1981).

1.2 - Quality and regulatory guidelines compliance

This study is conducted according to the Standard Operating Procedures of PhysioStim and to "GLP spirit" from the following texts:

- Bonnes Pratiques de Laboratoire, arrêté du 14 mars 2000 (Journal Officiel du 23 mars 2000), Fascicule spécial n° 2000/5 bis, Ministère de l'Emploi et de la Solidarité.
- Directive 2004/10/CE du Parlement Européen et du Conseil du 11 février 2004 (Journal Officiel du 20 février 2004).
- OECD Principles of Good Laboratory Practices, as revised in 1997.
- Decree n°2001-486 6 June 2001 of Ministère des Affaires Etrangères relative to the protection of animals used for experimental purposes (Official Journal 8 june 2001).
- The OECD 403 guideline for testing of chemicals "Acute Inhalation Toxicity, 12 May 1981.

2 - EXPERIMENTAL PROCEDURE

2.1 - Animals

2.1.1 - Animals characteristics

Species: Rat
Strain: OFA (Sprague Dawley)
Supplier: Grimaud Frères (Roussay, France) or Charles River Laboratories (L'Arbresle, France)
Sex: female and male
Identification: no specified when the study plan is being prepared
Weight for the ordered animals: 140 to 250 g
Weight on the test day: 170 to 320 g
Animals house codes: no specified when the study plan is being prepared
Number of animals per experiment: 24 rats (12 females and 12 males)
Number of animals to be ordered: 24 rats (12 females and 12 males)

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The females are nulliparous and non-pregnant.
The weight variation in animals or between groups used in this test must not exceed +/- 20 % of the mean weight.

2.1.2 - Housing

At their arrival, rats are submitted to clinical examination and excluded if they present any sign of bad health.
They are acclimatized to laboratory conditions for at least 5 days.
They are housed at PhysioStim facility, in cages (1032 cm^2), cleaned twice a week, with air-conditioning (20 to 25°C), relative humidity (30 to 70%), artificial day/night cycle: 12h/12h (light on at 9h00) as described in the corresponding current standard operating procedure.

2.1.2.1 Husbandry & equipment

The animals are tested with inhalation equipment designed to sustain a dynamic air flow of 12 to 15 air changes per hour, ensure an adequate oxygen content of about19% and an evenly distributed exposure system (Appendix 5)
The design of the chamber used minimises crowding of the test animals and maximises their exposure to the test substance. To ensure stability of a chamber atmosphere, the total "volume" of the test animals is about 5% of the volume of the test chamber.
A dynamic inhalation system with a suitable analytical concentration control system is used.
The observation period should be determined reference to the behaviour during the test.
The day of the experiment, the rats are transferred in the experimental room at a ratio of 2 animals per cage.
Animals will be group-caged by sex.

2.1.2.2 Diet and water

Before the test:
• Diet: For feeding, conventional laboratory diets may be used.

• Water: unlimited supply of drinking water may be used, municipal tap water for human consumption *ad libitum*.
Bacterial and chemical analyses of water are performed regularly by external laboratories.

Food is withheld during exposure whereas water may also be withheld in certain cases.

2.2 - Test article, vehicle and reagents

2.2.1 - Test article

2.2.1.1 Raw material

Denomination: NORASYSTEM®

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Supplier: PHODÉ
Appearance: Clear liquid, orange-coloured
Apparent density: 1.00 to 1.10
pH (21°C pure): 8.80 ± 0.50
Retest date: not available
Storage conditions: Store the product in dry environment and protect it from rays of light.

2.2.1.2 Formulation

Drug storage conditions, chemical and physical properties are supplied by PHODE. At the receipt of the reference article, it is registered and stored under specified conditions.

2.2.2 - Vehicle

2.2.2.1 Raw material

The test article (distilled water) is supplied by MILLIPORE system ELIX.

2.2.3 - Reagents

2.2.3.1 Anaesthetic agent

2.2.3.1.1 Raw material

Denomination: sodium pentobarbital
Supplier: CEVA Santé Animale
Pharmaceutical form: injectable preparation
Batch number: not available
Appearance: clear colorless liquid
Expiry date: not available
Storage conditions: ambient (or +4°C before use)

2.3 - Study design

2.3.1 - Acute administration of test article

Before the experimentation, the rats are weight and put in the experimental cage for an acclimatising period of one bour.
Then, they are exposed to the test concentration in the designated apparatus for 4 hours, one concentration being used per group.
When a vehicle is used to generate an appropriate concentration of the substance in the atmosphere, a vehicle group is used.
Subsequently, observations of effects and deaths are performed (using a webcam). The observation period is 2 days.
Animals which die during the test are insinerated (FERSO BIO), and at the conclusion of the test, surviving animals are sacrificed with an intraperitoneal injection of a high dose of sodium pentobarbital.

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2.3.2 - Test description and general organization of the experiment

- before the test, animals are randomized and assigned to the number of groups.
- the day of experiment, rats are differenciated by a mark, in order to facilitate the analysis of the behaviour during exposure and after during the recovery time.

Indeed, in a group, each rat are identified by a different mark (color or form) on its head to distinguish themselves during the exposure.

2.3.3 - Specific apparatus

The volatile substance is exposed at a concentration of 5 mg/L for 4 hours.
If this test produces no compound-related mortality, then a full study three dose levels may not be necessary.
For this study, the specific apparatus is described as follows:
System: "Dry vapour" diffuser (venturi nebulizer system) appendix 5
Cage: appendix 3
WebCam: Microsoft LifeCam VX-6000

2.3.4 - Administration of test, control article

This study includes 4 groups:

- Group 1: male test article group n=6
- Group 2: female test article group n=6
- Group 3: male control article group n=6
- Group 4: female control article group n=6

Each group is composed of male (n=6) and female (n=6) rats.

2.3.5 - Clinical examination

During and following exposure, observations are made and recorded systematically; A careful clinical examination is made at least once each day.
Cageside observations includes, but are not limited to, changes in the skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behaviour pattern. Particular attention will be directed to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.
The time of the death is recorded as precisely as possible.
Individual weights of animals are determined daily after exposure, and at death. Changes in weight should be calculated and recorded when survival exceeds one day. At the end of the test the surviving animals are weighed and sacrificed as described in § 2.3.1.
All the clinical details of each rat are reported on an individual clinical sheet (appendix 4).

2.4 - Data & Reporting

Data may be summarised in tabular form showing for each test group the number of animals at the start of the test, time of death of individual animals at different exposure levels, number of animals displaying other signs of toxicity.

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The final report includes the following information:

- Test conditions as described in the overall previous § (from §1 to § 2.3.5)
- Ratio of death per group: the time of death is recorded and general observation is done during and after the exposure.
- Different observations during and after exposure
- A video recording of the behaviour during the period of exposition.

Both a paper version and a computing version of this file will be transmitted to PHODÉ.

3 - STATISTICAL ANALYSIS

A statistical analysis will be performed at the end of the experiments.

4 - ARCHIVING

The sample of test and reference article is stored until its respective retest date. The study file is stored for, at least, 5 years from the end of the study in the archives of PhysioStim:

PhysioStim
Z.I. de Brénas
81440 Lautrec, France
At the end of this retention period, PhysioStim will contact the Sponsor to know the new location of archive.

The study file includes:

- The study plan and possible amendments
- The individual data (including CD-Roms)
- A tabular format
- The statistical analysis
- The final report
- The correspondence

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APPENDICES

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Appendix 1: Certficate of analysis

ALBI, on the 08th February 2007

CERTIFICATE OF ANALYSIS

PRODUCT: NORASYSTEM®

BATCH N°:
Manufactured on :

	PHYSICAL CONSTANTS	ANALYSIS
APPEARANCE	Liquid orange	T
ODOUR	Orange	T
COLOUR liquid	Orange	N.S.
Colour at 10 % dilution	Colourless	N.S.
DENSITY at 20 °C	1,00 to 1,10	T
pH (21 °C pure)	8,80 +/- 0,50	T

T : True to the reference

RESULT:

CONFORM

COMMENT :

THE PRODUCT IS IN CONFORMITY WITH THE SAMPLE OF REFERENCE.

*** ANCSC : analysis non carried out with standard checks**
*** NA : NON APPLICABLE**
*** NS : NON SIGNIFICANT**

IN CHARGE OF QUALITY

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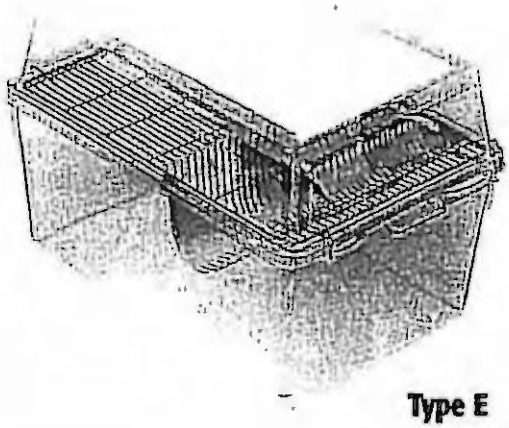
Appendix 2: Conditionning system



Type E

- Matériaux :
  - enveloppe en PET transparent,
  - filtre polyester non tissé Reemay,

  son utilisation diminue considérablement le risque de contamination croisée et d'allergie pour le personnel, les couvercles S, M, E, II, III ne sont pas autoclavables ; ils peuvent être décontaminés par le formol gazeux, les vapeurs d'acide péracétique ou être irradiés.
  Le couvercle FI est autoclavable (121°C - 20 min.).
- Applications :
  - hébergement de lots différents d'animaux dans un même local,
  - hébergement d'animaux de provenance différente dans un même local,
  - hébergement d'animaux immunodéprimés (ex : souris Nude) ou fragilisés (ex : animaux opérés),
  - maintien du statut sanitaire IOPS, SSC[tm], ...
  - réduction très importante des projections de litière et d'aliment, de poils et de particules générés par les animaux (allergie du personnel).
- Certaines applications nécessitent le respect de procédures rigoureuses (ex : utilisation de hotte à flux laminaire) ou autres systèmes de confinement (ex : enceinte).

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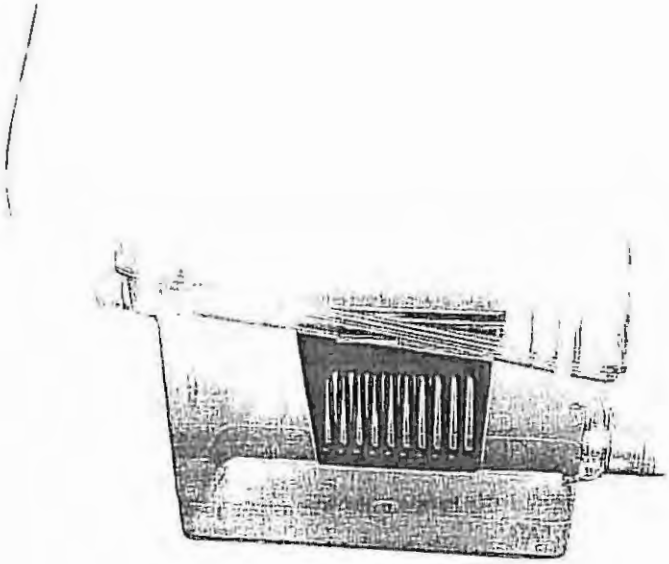
Appendix 3: Experimental system



- Dimensions intérieures (mm) :
  profondeur 324, largeur 163, hauteur 148 x 190.
- Dimensions hors tout (mm) :
  profondeur 389, largeur 204, hauteur 241
- Surface au sol : 528 cm².
- Description :
  cage en polycarbonate transparent,
  autoclave = 121C pendant 20 minutes.

- *Internal dimensions(mm) :*
  depth 324, width 163, height 148 to 190.
- *Overall dimensions (mm) :*
  depth 389, width 204, height 241.
- *Floor area : 528 cm².*
- *Description :*
  cage in transparency polycarbonate,
  autoclavable at 121 C for 20 minutes.

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Appendix 4: Clinical sheet

CLINICAL SHEET

Race: **Rat** Sex: ☐ M ☐ F Arrival date:

Identification number

RECEPTION CLINICAL EXAM		WEIGHT:
General behaviour	☐ Tonic	☐ Stagnant
Hairs	☐ Shiny	☐ Dull
Diarrhoea	☐ Presence	☐ Absence
Nasal Discharge	☐ Presence	☐ Absence
Ocular discharge	☐ Presence	☐ Absence
Respiratory frequency	☐ High	☐ Low
Swollen genital glands	☐ Yes	☐ No
Wink of eyes	☐ High	☐ Low
CONCLUSION		
Animal accepted	☐ Yes	☐ No ☐ Return to supplier ☐ Euthanasia

Date	Name	Visa

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FOLLOW-UP CARE OF ANIMAL		
Date	Description	Visa
OBSERVATION PHASE CLINICAL EXAM DAY 0: EXPERIMENTATION		WEIGHT:
Time at which signs of toxicity appear : Time of exposure before death:		
CONCLUSION		

Date	Name	Visa

OBSERVATION PHASE CLINICAL EXAM DAY 1		WEIGHT:
General behaviour	☐ Tonic	☐ Stagnant
Changes in skin	☐ Yes	☐ No
Changes in fur	☐ Yes	☐ No
Changes in mucovis membranes	☐ Yes	☐ No
Convulsions	☐ Yes	☐ No
Tremors	☐ Yes	☐ No
Salivation	☐ Yes	☐ No
Lethargy	☐ Yes	☐ No
Coma	☐ Yes	☐ No
Hairs	☐ Shiny	☐ Dull
Diarrhoea	☐ Presence	☐ Absence
Drowsiness	☐ Presence	☐ Absence
Nasal Discharge	☐ Presence	☐ Absence
Ocular discharge	☐ Presence	☐ Absence
Changes in respiratory	☐ Yes	☐ No
Wink of eyes	☐ High	☐ Low
Death	☐ Yes	☐ No

Date	Name	Visa

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OBSERVATION PHASE CLINICAL EXAM DAY 2		WEIGHT:
General behaviour	☐ Tonic	☐ Stagnant
Changes in skin	☐ Yes	☐ No
Changes in fur	☐ Yes	☐ No
Changes in mucovis menbranes	☐ Yes	☐ No
Convulsions	☐ Yes	☐ No
Tremors	☐ Yes	☐ No
Salivation	☐ Yes	☐ No
Lethargy	☐ Yes	☐ No
Coma	☐ Yes	☐ No
Hairs	☐ Shiny	☐ Dull
Diarrhoea	☐ Presence	☐ Absence
Drowsiness	☐ Presence	☐ Absence
Nasal Discharge	☐ Presence	☐ Absence
Ocular discharge	☐ Presence	☐ Absence
Changes in respiratory	☐ High	☐ Low
Wink of eyes	☐ High	☐ Low
Wink of eyes	☐ High	☐ Low
Death	☐ Yes	☐ No

Date	Name	Visa

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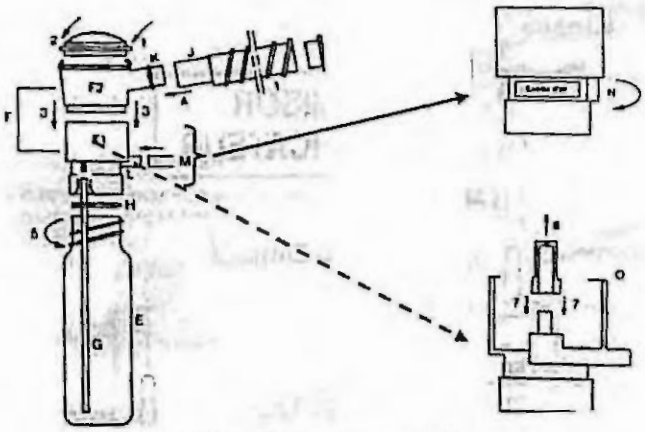
Appendix 5: Characteristics and description of diffuser

This apparatus allows the diffusion of "dry vapour", that is to say it generates a mist of particles of very small size.



Representation of the venture nebuliser system

This device ensure to keep a relative humidity level between 30% and 75%; and to deliver inside each cage an air flow in accordance with the specifications of the OECD 403 guideline (19 air changes by hour – compressor air flow = about 2.85 L/min/cage).

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Appendix 2: Individual weights of rats

rat identification	group	reception day (J-6 for vehicle groups) (J-7 for treated groups)	Day of experiment (J)	J + 1	J + 2	DELTA (J+1 vs J)	DELTA (J+2 vs J)
1	vehicle male	200	240	240	258	0	18
2	vehicle male	196	250	256	276	6	26
3	vehicle male	202	260	250	278	-10	18
4	vehicle male	204	250	254	268	4	18
5	vehicle male	198	240	248	258	8	18
7	vehicle male	200	240	240	258	0	18
moy	vehicle male	200.0	246.7	248.0	266.0	1.3	19.3
sem	vehicle male	1.2	3.3	2.8	3.8	2.6	1.3
6	treated male	202	244	252	254	8	10
8	treated male	198	250	248	252	-2	2
9	treated male	202	260	272	280	12	20
10	treated male	198	258	270	276	12	18
11	treated male	198	260	272	278	12	18
12	treated male	200	258	272	274	14	16
moy	treated male	199.7	255.0	264.3	269.0	9.3	14.0
sem	treated male	0.8	2.7	4.6	5.1	2.4	2.8
1	vehicle female	154	184	190	186	6	2
2	vehicle female	144	184	180	182	-4	-2
3	vehicle female	140	170	180	178	10	8
4	vehicle female	140	190	190	190	0	0
5	vehicle female	138	170	174	168	4	-2
7	vehicle female	144	172	176	182	4	10
moy	vehicle female	143.3	178.3	181.7	181.0	3.3	2.7
sem	vehicle female	2.3	3.6	2.8	3.1	2.0	2.1
6	treated female	140	178	182	178	4	0
8	treated female	142	178	192	196	14	18
9	treated female	142	180	190	188	10	8
10	treated female	140	174	172	170	-2	-4
11	treated female	140	188	192	192	4	4
12	treated female	142	172	178	180	6	8
moy	treated female	141.0	178.3	184.3	184.0	6.0	5.7
sem	treated female	0.4	2.3	3.4	4.0	2.3	3.1

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Appendix 3: Identification code of cages and perfusion system

Male group

Mark on the tail	Rat N°
1 Red line	1
2 Red line	2
3 Red line	3
1 blue line	4
2 blue line	5
3 blue line	6
1 black line	7
2 black line	8
3 black line	9
1 long Red line	10
1 long blue line	11
1 long black line	12

Female group

Mark on the tail	Rat N°
1 Red line	1
2 Red line	2
3 Red line	3
1 blue line	4
2 blue line	5
3 blue line	6
1 black line	7
2 black line	8
3 black line	9
1 long Red line	10
1 long blue line	11
1 long black line	12

Cage 1 of experiment
Cage 2 of experiment
Cage 3 of experiment