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STUDY / PRODUCT REFERENCES : Tj 290 / 03-2070

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ACUTE ORAL TOXICITY TEST IN THE RAT
- Acute toxic class method -
TEST SUBSTANCE : ING 911

TEST REPORT

Blanquefort, July 17, 2003

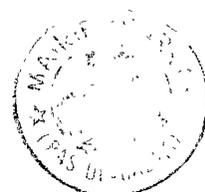
13 pages in this report including 4 in Appendices

PG/MCC

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- Acute toxic class method -
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EVALUATION DE LA TOXICITE AIGUE APRES ADMINISTRATION PAR VOIE ORALE CHEZ LE RAT – METHODE PAR CLASSE DE TOXICITE AIGUE - LIGNE DIRECTRICE 423 DE L'OCDE (17/12/2001).

ACUTE ORAL TOXICITY TEST IN THE RAT - ACUTE TOXIC CLASS METHOD - OECD GUIDELINE 423 (17/12/2001).

• **Substance testée / Test substance :**

ING 911 – lot/ batch 06/03

• **Résumé de l'essai / Summary of the test :**

L'objectif de l'essai a été d'apprécier qualitativement et quantitativement les phénomènes toxiques et le délai de leur apparition après administration unique d'une dose prédéterminée de 2000 mg/kg de poids corporel, de la substance à tester diluée dans l'eau PPI, par voie orale, chez 6 rats femelles, selon un processus séquentiel.

Les animaux ont été observés quotidiennement pendant 14 jours au moins après l'administration et les signes de toxicité dont la mortalité ont été notés.

Le test a fourni des résultats permettant de classer la substance à tester, dans le Système de classification Globalement Harmonisé (SGH) de substances entraînant de la toxicité aiguë (OCDE 1998).

The aim of the study was to assess qualitatively and quantitatively the toxic effects and the delay of appearance after single oral administration of pre-defined dose of 2000 mg/kg of body weight, of test substance diluted with distilled water, in 6 female rats, using a stepwise procedure.

The animals were daily observed for at least 14 days after administration and the signs of toxicity (mortality...) were noted.

This test provided results allowing the test substance to be ranked and classified according to the Globally Harmonised System (GHS) for the classification of chemicals which causes acute toxicity (OECD 1998).

• **Dates de l'expérimentation/ Experimental dates :** du/ from 30/06/03 au/ to 16/07/03

• **Résultats/ Results :**

0 % de mortalité à la dose de 2000 mg/kg.
0 % of mortality at the dose of 2000 mg/kg.

• **Conclusion/ Conclusion :**

La substance testée a été classée dans la **Catégorie de danger 5 ou non classée** avec une **DL50 supérieure à 2000 mg/kg.**

The test substance was classified in the hazard Category 5 or unclassified with a LD50 higher than 2000 mg/kg.

I. AIM AND PRINCIPLE OF THE TEST



The aim of the test is to obtain, using a stepwise procedure with a minimum number of animals per step, sufficient information on the acute toxicity after single administration, by oral route in the Rat, of a test substance, for its classification.

The test substance is administered to a group of experimental animals, by oral route (gavage), at one of the defined doses (5 mg/kg, 50 mg/kg, 300 mg/kg or 2000 mg/kg) according to the available information on the test substance.

The substance is tested using a stepwise procedure, each step using 3 animals maximum of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step, will determine the next step (no further testing, or dosing of additional animals with a same, higher or lower dose level).

The animals are observed each day for 14 days at least, after administration, to detect the signs of toxicity. Animals which die are necropsied ; the animals which are intercurrently sacrificed as well as the ones which survive until the end of the test are necropsied.

Rat is the Rodent species commonly used and recommended by official authorities for the assessment of chemical substances safety by this type of method.

This test using pre-defined doses provides results allowing the test substance to be ranked according to the Globally Harmonised System (GHS) for classification of chemicals which cause acute toxicity (OECD 1998).

II. REFERENCES

- The OECD guideline 423 of December 17, 2001 concerning the tests of chemical products.
- OECD (1998) - Harmonized Integrated Hazard Classification System For Human Health And Environmental Effects Of Chemical Substances - as endorsed by - the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, November 1998, Part 2, p. 11 [<http://webnet1.oecd.org/oecd/pages/home/displaygeneral/0,3380,EN-documents-521-14-no-24-0o-0,FF.html>]

III. TEST FACILITY

III.1. Test facility and technical staff

EVIC France – Division Evic-Tox
48 rue Jean Duvert
33290 BLANQUEFORT
05 56 95 59 95

Study director : Patrick GOMOND
Responsible technician : Martine MIERMON

III.2. Ethics and approval of the Test Facility

The test was entirely performed according to the animal ethical rules mentioned in the **European Directive 86/609/EEC** of November 24, 1986 and was submitted to the previous agreement of the animal Ethics Committee internal to the Test Facility.

It was conducted according to the internal rules of the Quality System of EVIC france company, which was declared in conformity with the GLP by the **AFSSAPS** (decree of March 14, 2000

published in the Official Journal of the French Republic of March 23, 2000) and the **GIPC** (decree No 98-1312 of December 31, 1998 published in the Official Journal of the French Republic of January 1st, 1999) and with the NF EN ISO/CEI 17025 standard by the **COFRAC** (accreditation No 1-0042).

IV. QUALITY ASSURANCE

All the data collected during the test were recorded by the technician responsible for the test, on the documents reserved for that effect.

Each page of these documents was initialled and dated by the technician responsible for the test. Any missing data was justified and the corrections were initialled and dated.

The Quality Assurance Unit ensured by periodic audits that the study plan and working procedures relevant to this type of test were strictly applied. Any modification to the study plan is submitted to an amendment signed by the Sponsor and the Study Director.

The Quality Assurance Unit performed regular audits about the Test Facility in compliance with the corresponding procedure.

The experimental data and the test report were audited in accordance with the procedure implemented in the Test Facility.

At the end of the test, the work documents were filed with the test report for 10 years in the filing room of the Test Facility.

At the end of this period, the Test Facility defines with the Sponsor the carrying out of the filing, the restitution of the data or their destruction.

V. DEFINITION OF THE TEST SUBSTANCE

V.1. Substance reference

The Sponsor provided for the test 1 bag containing approximately 500 g of the substance identified as : **ING 911 – batch 06/03**.

It was a white powder.

This substance was coded under the reference 03-2070.

V.2. Storage

The test substance was stored at ambient temperature and out of the light in a room especially fitted out to that effect.

A sample of the test substance was filed in the samples library of the Test Facility as a reference where it will be kept until its expiry date or for a maximum period of 10 years.

VI. REACTIVE SYSTEM

Species : SPF (specific pathogen free) Sprague-Dawley albino rats



Origin : CHARLES RIVER Laboratories (69210 L'Arbresle, France)

Age : about 7 weeks (when put in acclimatization)

Number and sex : 6 nulliparous and non-pregnant females

Acclimatization : for at least 5 days prior to the test

Weight : on D-1, the day before the corresponding step of the test, animals were weighed. The mean weight was calculated and the acceptable limits were deduced, the extreme individual weights of the animals could not deviate from the mean weight by more than $\pm 20\%$. If the weight of an animal exceeded the authorized limit, the Study Director was warned in order to decide to keep it for the test.

Identification : the animals were identified individually per cage maximum, by marking with picric acid : the location of the marking, different for each animal, corresponded to a number. A caudal marking represented by a coloured circle with a marker pen enabled to identify the group.

Housing : the animals were housed at the rate of 3 per cage maximum, in 31 cm x 46 cm x 19 cm polypropylene cages with stainless steel lid. The bedding renewed regularly, was composed of wood shavings delivered dust-free and sterilized to γ radiations. It was supplied by SICSA (94142 Alfortville, France).

The cages were placed in limited-access premises, maintained in slight overpressure (a minimum of 10 mm of water), under air-conditioned temperature ($t = 22 \pm 2^\circ\text{C}$) and controlled relative humidity ($\text{RH} = 50 \pm 20\%$) except during washing cycles and whose renewal in fresh filtered air (on absolute filter) was performed at the rate of about 10 cycles per hour. The artificial lighting ensured a sequence of 12 hours light, 12 hours dark.

Feeding : the complete diet was supplied under pelleted form A04-10, delivered sterilized to γ radiations by UAR (91360 Epinay sur Orge, France).

Drinking : the acidified tap water ($\text{pH} = 2.5$) was distributed in polypropylene bottles with stainless steel teat. A sample of water was taken after each technical intervention from the pipes and every 6 months at least and sent for chemico-physical and bacteriological analysis to a specialized control organization.

VII. TEST PROCEDURE

VII.1. Preparation of the test substance

The test substance was administered diluted with distilled water (Cooper – batch 0007) after being homogenized by manual agitation and maintained under magnetic stirring during treatment.

The vehicle was chosen according to the nature of the test substance and producing no painful effect in disagreement with the rules of animal ethic.

The preparation of the substance was performed extemporaneously the day of the test D1/T0 in sufficient quantity for the necessities of the test. The method of preparation was reported in the work document reserved for that effect.

The test substance was brought to the test room according to the modalities defined in the procedure of the Test Facility.



VII.2. Experimental chronology

According to the available information about the toxicity of the test substance, the test started on 3 animals (Step 1) receiving a dose of **2000 mg/kg** of body weight of test substance.

After this 1st step, according to the methodology described in the Annex 2d of the OECD guideline 423 (joined in Appendix IV of the present report), the test was performed with 3 animals receiving the test substance at the dose level of **2000 mg/kg** of body weight, under the same conditions as the animals from the step 1.

The experimental procedure, similar for every step, was described in the following chapters.

VII.3. Administration of the test substance (D1/T0)

The animals, fasted prior to the test substance administration, were weighed again on D1 before administration.

The volume per kg of body weight, defined to **20 ml/kg**, the volumes of suspension to administer were calculated for each rat.

The test substance was administered in a single dose, orally, by gavage using a syringe with appropriate volume, fitted with a suitable sized canula.

After administration, animals were fasted for 3 to 4 hours.

VII.4. Body weight

The animals were regularly weighed on D-1 the day before administration then on D1/T0 just before administration of the substance and on D4, D8 and D15 i.e. 3, 7 and 14 days after administration of the test substance.

The results of the weighings were reported in the work document reserved for that effect.

VII.5. Clinical observations

Animals were regularly observed the day of administration (immediately, during the 30 minutes following gavage, 1h, 2h, 3h and 4h after administration) then at least once a day for 14 days at least.

Just after administration of the test substance, the attention was directed to suffocation signs : in case of mortality, dead animals were necropsied immediately and it was checked that the death was related to the toxicity of the test substance and not to an error of route.

Systematically, since the appearance of the clinical signs and at the latest, 30 minutes after administration, the clinical observations were made individually, each animal being examined outside the home cage.

At the other control times, the examinations were performed cageside, without touching the animals. Any animal which had an abnormal behaviour was taken out from its cage and submitted to an individual examination.

The different parameters observed were the following ones : spontaneous activity, Preyer's reflex, respiratory effect, convulsions, tremors, temperature, muscular tone, grip strength, palpebral ptosis, mydriasis, salivation, lacrimation, turnaround reflex, piloerection, diarrhoea, lethargy, coma, changes in skin, fur, eyes, mucous membranes and death.



The results of the clinical observations were registered in the work document reserved for that effect.

VII.6. Necropsy

All the animals surviving at the end of the 14 days of observation were sacrificed on D15 by intraperitoneal injection of a 6 % sodium Pentobarbital® solution at the rate of 1.16 ml/kg and bled at the femoral artery. They were necropsied and the main organs were examined macroscopically.

All the pathological changes were recorded in the document reserved for that effect.

VII.7. Interpretation of the results

The assessment criteria of the toxicity of the test substance taken into account were :

- body weight change
- clinical and behavioural signs
- necropsy findings
- the mortality expressed in percentage of compound-related deaths.

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, in compliance with the GHS (Appendix IV) :

- Category 1 = 0 < LD50 ≤ 5 mg/kg
- Category 2 = 5 mg/kg < LD50 ≤ 50 mg/kg
- Category 3 = 50 mg/kg < LD50 ≤ 300 mg/kg
- Category 4 = 300 mg/kg < LD50 ≤ 2000 mg/kg
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified

VIII. RESULTS

The individual weights of the animals from the 2 groups assessed during the test are supplied in Appendix I.

The results of the clinical observations are summarized in the table enclosed in Appendices II.

The results of the necropsy findings are summarized in the table enclosed in Appendix III.

IX. CONCLUSION

According to the Globally Harmonised System (GHS) for the classification of substances which cause acute toxicity, the substance **ING 911** was classified in the **hazard category 5 or unclassified** with a **LD50** higher than **2000 mg/kg** in the Rat.

X. STUDY RESPONSIBLE PERSONNEL'S STATEMENT

Study Director

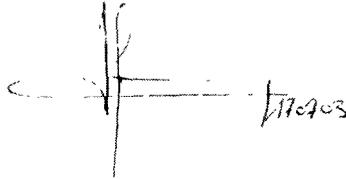
I the undersigned, **Patrick GOMOND**, declare that the overall conduct of the study was carried out under my responsibility and in accordance with the principles of Good Laboratory Practices according to the rules appropriate to this study.



X. STUDY RESPONSIBLE PERSONNEL'S STATEMENT

Study Director

I the undersigned, **Patrick GOMOND**, declare that the overall conduct of the study was carried out under my responsibility and in accordance with the principles of Good Laboratory Practices according to the rules appropriate to this study.



A handwritten signature in black ink, appearing to read 'Patrick Gomond', with a horizontal line extending to the right.

Quality Assurance

I the undersigned, **Danièle PICARD**, declare that :

- this kind of study was audited according to the procedure of the Test Facility on July 1st, 2003,
- the report of the audit was transmitted to the Management and to the Study Director on July 7, 2003,
- the final report was audited on July 21, 2003,
- the results reported accurately and completely reflect the raw data of the study.



A handwritten signature in black ink, appearing to read 'Danièle Picard', with the date '24 07 03' written below it.



Body Weight

Dose = 2000 mg/kg

Animals No		Weight (in g)				
		D1	D4	D8	D15	D15-D1
Step 1	9556	204.7	231.8	239.7	250.6	45.9
	9557	207.9	234.7	243.5	259.2	51.3
	9558	207.1	231.8	234.4	254.9	47.8
Step 2	9559	222.4	243.7	249.9	263.5	41.1
	9560	211.5	232.6	243.9	247.8	36.3
	9561	216.5	239.6	248.2	264.8	48.3
Mean		211.7	235.7	243.3	256.8	45.1
standard deviation		6.7	4.9	5.7	6.9	5.5



Appendix II

Clinical observations

Dose = 2000 mg/kg

Observation time	Comments	Observation time	Comments
D1 (after treatment)	Slight piloerection	D6	NTR
D1 T30'	Slight piloerection	D7	NTR
D1 T1h	Slight piloerection	D8	NTR
D1 T2h	NTR	D9	NTR
D1 T3h	NTR	D10	NTR
D1 T4h	NTR	D11	NTR
D2	NTR	D12	NTR
D3	NTR	D13	NTR
D4	NTR	D14	NTR
D5	NTR	D15	NTR

NTR : nothing to report



Necropsy

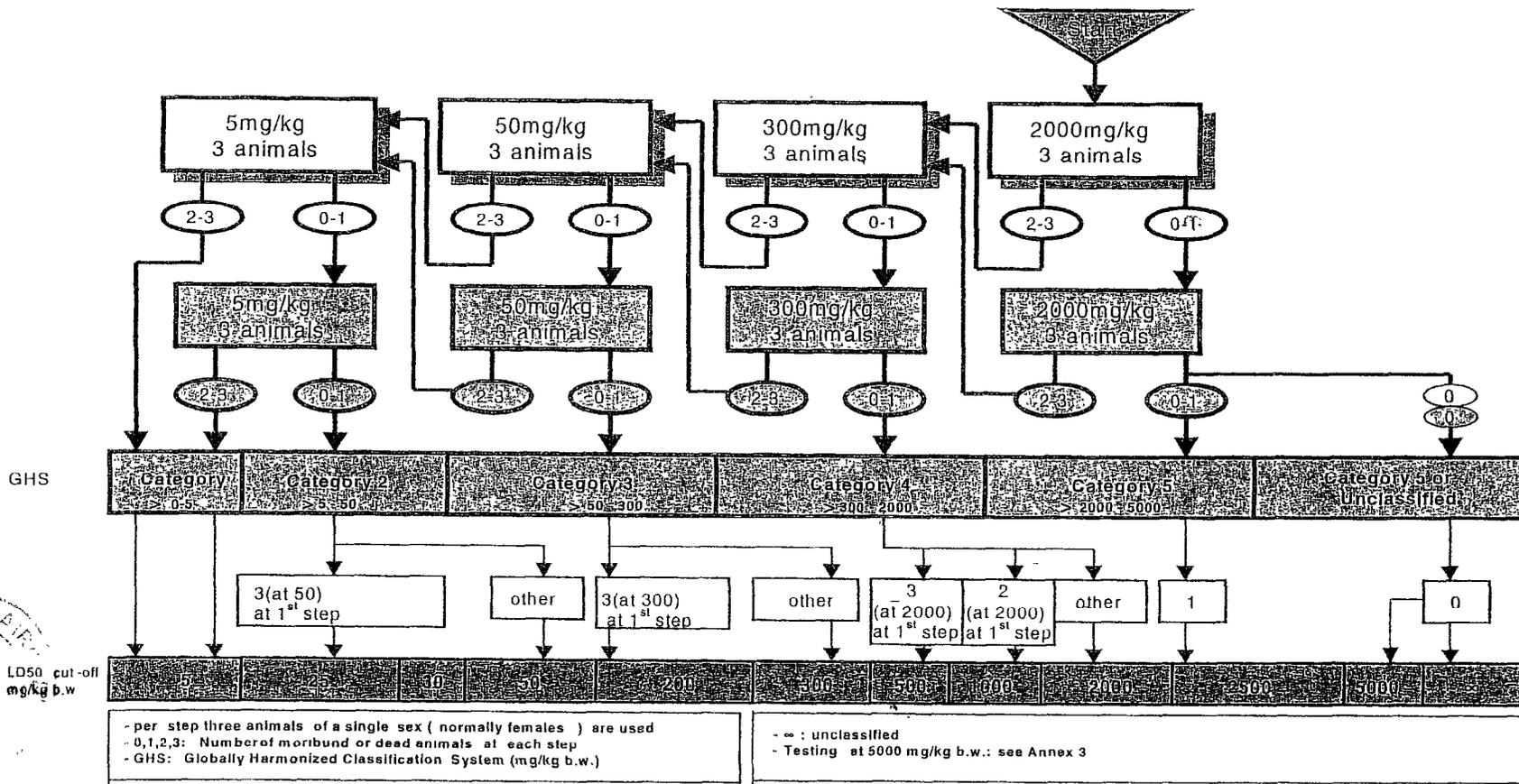
Dose = 2000 mg/kg

Animals No		Death		Comments
		Day	Reason	
Step 1	9556	D15	S	NTR
	9557	D15	S	NTR
	9558	D15	S	NTR
Step 2	9559	D15	S	NTR
	9560	D15	S	NTR
	9561	D15	S	NTR

NTR = nothing to report
ND = natural death
S = sacrifice
AD = accidental death



ANNEX 2d: TEST PROCEDURE WITH A STARTING DOSE OF 2000 MG/KG BODY WEIGHT



Appendix IV

Study ref. : Tj 290 / 03-2070