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NOTOX B.V.			
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INITIAL SUBMISSION: LETTER FROM ELF ATOCHEM NA INC TO USEPA RE FINAL REPORTS OF SENSITIZATION AND PRIMARY IRRITATION STUDIES W/T-BUTYLPEROXYACETATE, *, W/ATTCHMTC & DATED 3/31/1999			
Chemical Category			
T-BUTYLPEROXYACETATE; T-BUTYLPEROXYPIVALATE; T-BUTYLPERO*; *			

INITIAL  
SUB-  
MISSION

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8EHQ-0999-14545

**elf atochem**



Elf Atochem North America, Inc.  
2000 Market Street  
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Washington, D.C. 20460



8EHQ-99-14545

~~Confidential~~

Subject: TSCA Section 8(e) Submission

Dear Sir/Madam:

Elf Atochem North America Inc. (Elf Atochem) is submitting final reports for two contact sensitization studies in guinea pigs, one primary skin irritation study and one eye irritation study in rabbits to the Environmental Protection Agency (EPA) pursuant to Toxic Substances Control Act (TSCA) Section 8(e). These studies provide information on several compounds as noted below. They do not involve effects in humans.

Nothing in this letter or the attached study reports are considered confidential business information of Elf Atochem.

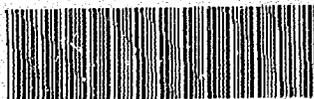
In guinea pig maximization tests, sensitization rates of 80% and 89% were reported for t-butylperoxyacetate (CAS No. 107-71-1) and t-butylperoxypivalate (CAS No. 927-07-1), respectively. The results of a primary skin irritation study with t-butylperoxy isopropyl carbo.ate (CAS No. 2372-21-6) showed the material to be severely irritating to rabbit skin after a 4-hour exposure. Thioacetic acid (CAS No. 507-09-5) was severely irritating to rabbit eyes.

Further questions regarding this submission may be directed to me at (215) 419-5890.

Sincerely,

*Debra Randall*

Debra Randall, DABT  
Product Safety Manager



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REPORT

ASSESSMENT OF CONTACT HYPERSENSITIVITY TO  
TERT-BUTYLPEROXYPIVALATE 75% IN AROMATIC FREE MINERAL SPIRIT

IN THE ALBINO GUINEA PIG

(MAXIMISATION-TEST)

*tert-Butylperoxy pivalate*  
*CAS # 927-07-1*

NOTOX Project 245194  
NOTOX Substance 84294

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TERT-BUTYLPEROXYPIVALATE 75%  
IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245194

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CONFIDENTIALITY STATEMENT

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TERT-BUTYLPEROXYPIVALATE 75%  
IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245194

STATEMENT OF GLP COMPLIANCE

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NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

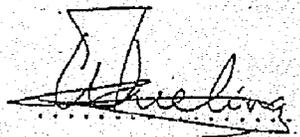
Japanese Ministry of Agriculture, Forestry and Fisheries (59 NohSan, Notifications No. 3850).

Japanese Ministry of International Trade and Industry (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85).

Study Director:  
Drs. A.H.B.M. van Huygevoort

Management:  
Drs. W.J.A.M. Frieling

  
Date: 26 February 1999

  
Date: 26 February 1999

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TERT-BUTYLPEROXYPIVALATE 75%  
IN AROMATIC FREE MINERAL SPIRIT

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QUALITY ASSURANCE STATEMENT

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NOTOX B.V., 's-Hertogenbosch, The Netherlands

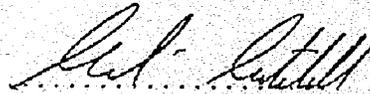
This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below. During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/ AUDITS	REPORTING DATES
on-site inspection(s)	
20 October 1998	20 October 1998
18 November 1998	18 November 1998
protocol inspection(s)	
9 October 1998	9 October 1998
report audit(s)	
13 January 1999	13 January 1999

Head of Quality Assurance:

C.J. Mitchell B.Sc.



Date: 4-3-99.

## SUMMARY

Assessment for Contact Hypersensitivity to TERT-BUTYLPEROXYPIVALATE 75% IN AROMATIC FREE MINERAL SPIRIT in the Albino Guinea Pig (Maximisation Test).

The study was carried out based on the guidelines described in: EC Commission Directive 96/54/EC, Part B.6, "Skin Sensitisation" and OECD No. 406, "Skin Sensitisation", and based on the method described by Magnusson and Kligman, "Allergic Contact Dermatitis in the Guinea Pig - Identification of Contact Allergens".

Test substance concentrations selected for the Main study were based on the results of a preliminary study.

In the Main study, experimental animals were intradermally injected with a 10% concentration and epidermally exposed to the undiluted test substance. Control animals were similarly treated, but with the vehicle (Corn oil) only. Two weeks after the epidermal application all animals were challenged with a 20% test substance concentration and the vehicle.

Skin reactions varying between grades 1 and 2 were observed in eight experimental animals in response to the 20% test substance concentration, 24 and/or 48 hours after exposure.

A skin reaction of grade 1 was observed in one control animal in response to the 20% test substance concentration, 48 hours after exposure.

One experimental animal was found dead on day 8. Macroscopic post-mortem examination of the animal showed dark red discoloration of the lungs and haemorrhages in the lungs and a reduction in size of the thymus. It was considered that the death of this animal was incidental and that the study outcome, based on the healthy surviving animals, was not adversely affected. No further mortality occurred and no further symptoms of systemic toxicity were observed in any of the animals of the main study.

Taking into account the occurrence and intensity of the responses and comparing these with the skin reactions seen in the control animals, it was considered that hypersensitivity to TERT-BUTYLPEROXYPIVALATE 75% IN AROMATIC FREE MINERAL SPIRIT had been induced in eight (of the nine) the experimental animals. The response seen in the control animal was considered to be a non specific skin reaction. These results indicate a sensitisation rate of 89 per cent.

Based on these results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), TERT-BUTYLPEROXYPIVALATE 75% IN AROMATIC FREE MINERAL SPIRIT should be labelled as: may cause sensitisation by skin contact (R 43).

PREFACE

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## Sponsors

Elf-Atochem S.A.  
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## Study Monitor

Mrs. Ir. W. M. Clous  
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NOTOX B.V.  
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The Netherlands

## Study Director

Drs. A.H.B.M. van Huygevoort

## Study Plan

Start : 19 October 1998  
End : 20 November 1998

TERT-BUTYLPEROXYPIVALATE 75%  
IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245194

TEST SUBSTANCE

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The sponsor is responsible for the completeness and GLP Compliance of all test substance data.

Identification	Tert-butylperoxypivalate 75% in aromatic free mineral spirit
Description	Clear colourless liquid
Akzo Nobel Trade Name	Trigonox 25-C75
Chemical Name	Tert-butylperoxypivalate 75% in aromatic free mineral spirit
Cas-No.	927-07-1
Batch	0419807135512
Purity	See Certificate of Analysis
Test substance storage	In freezer in the dark
Stability under storage conditions	Not indicated
Expiry date	01 October 1999 (allocated by NOTOX, 1 year after receipt of the test substance)
Density	approx. 850 kg/m <sup>3</sup> (20°C)
Stability in vehicle	Corn oil: at least 24 h
Vehicle	Corn oil
Rationale	The vehicle was selected based on a pretest performed at NOTOX.

To avoid exposure of the test substance to temperatures above -5°C as much as possible, the test substance was frozen in separate portions. Required amounts were defrosted immediately before use.

Preparation When required, the test substance formulations (w/w) were prepared prior to each treatment. No adjustment was made for specific gravity of vehicle. Homogeneity was obtained to visually acceptable levels.

PURPOSE AND RATIONALE

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The purpose of this study was to evaluate whether TERT-BUTYLPEROXYPIVALATE 75% IN AROMATIC FREE MINERAL SPIRIT induces contact hypersensitivity in guinea pigs after intradermal and epidermal exposure of the animals under the conditions described in this report.

This study should provide a rational basis for risk assessment in man. The Maximisation test is selected because it is regarded as the most sensitive and the preferred method with regard to testing for sensitisation potential.

## GUIDELINES

The study procedures described in this report were based on the following guidelines and test method:

European Community (EC), Council Directive 67/548/EEC, as last amended by Commission Directive 96/54/EC, Annex V, Part B, Methods for the Determination of Toxicity, B.6: "Skin sensitisation", Official Journal of the European Communities No. L 248, 1996.

Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No.406, "Skin Sensitisation", Paris Cedex, 1992.

"Allergic Contact Dermatitis in the Guinea-Pig: Identification of Contact Allergens" Magnusson B. Kligman A.M., 1970 published by C.C. Thomas, Springfield, Illinois, USA.

## ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years:  
raw data, protocol, report and test substance reference sample. No data will be withdrawn without the sponsor's written consent.

## TEST SYSTEM

Species	Dunkin Hartley strain, albino guinea pig (SPF-quality) Recognised by international guidelines as the recommended test system (e.g. OECD, EC). Source : Charles River, Germany.
Number of animals	Experimental group : 10 females. Control group : 5 females. (females were nulliparous and non-pregnant).
Age and body weight	Young adult animals (approx. 4 weeks old) were selected. Individual body weights did not exceed 500 grams.
Identification	Ear tattoo.
Reliability check	The results of a reliability test performed not more than 6 months previously are summarised in the Appendix. Similar procedures were used in the reliability test and in this study.

## ANIMAL HUSBANDRY

## Conditions

Air-conditioned room with approximately 15 air changes per hour and the environment controlled with optimal conditions considered as being a temperature of 21°C and a relative humidity of 50%. Fluctuations from these optimal conditions were noted, but were considered not to have affected study integrity. Lighting was 12 hours artificial fluorescent light and 12 hours dark per day.

## Accommodation

Group housing of 5 animals per labelled metal cage with wire-mesh floors and equipped with an automatic drinking system (ITL, Bergen, The Netherlands). The acclimatisation period was at least 5 days before the start of treatment under laboratory conditions.

TERT-BUTYLPEROXYPIVALATE 75%  
IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245194

#### Diet

Free access to standard guinea pig diet, including ascorbic acid (1600 mg/kg); LC 23-B, pellet diameter 4mm (Hope farms, Woerden, The Netherlands). Certificates of analysis were examined and retained in the NOTOX archives. In addition, hay (B.M.I., Helmond, The Netherlands) was provided once a week.

#### Water

Free access to tap-water, diluted with decalcified water. Certificates of quarterly analysis for tap-water were examined and retained in the NOTOX archives.

### PRELIMINARY IRRITATION STUDY

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A preliminary irritation study was conducted in order to select test substance concentrations to be used in the Main Study. The selection of concentrations was based on the following criteria:

- The concentrations are well-tolerated by the animals.
- For the induction exposures: the highest possible concentration that produced mild to moderate irritation (grades 2 - 3).
- For challenge exposure: the maximum non-irritant concentration.

The test substance concentrations used were from the series: Undiluted (if a liquid), 50%, 20%, 10%, 5%, 2%, 1% and, if needed, further lower concentrations using the same steps.

The test system, procedures and techniques were identical to those used during the main study, unless otherwise specified.

The animals were selected from stock and were between 5 and 9 weeks old, and as a consequence the body weights could exceed 500 grams. Body weights were determined prior to treatment.

#### Intradermal injections:

A series of four test substance concentrations was used; the highest concentration being the maximum concentration that could technically be injected. Each of two animals received two different concentrations in duplicate (0.1 ml/site) in the clipped scapular region. If possible, the injection sites were assessed for irritation 24 and 48 hours after treatment.

#### Epidermal application:

A series of four test substance concentrations was used; the highest concentration being the maximum concentration that could technically be applied. Two different concentrations were applied (0.5 ml each) per animal to the clipped flank, using Metalline patches<sup>†</sup> (2x3 cm) mounted on Medical tape<sup>†</sup>, which were held in place with micropore tape<sup>†</sup> and subsequently Coban elastic bandage<sup>†</sup>. The animals receiving intradermal injections were treated with the lowest concentrations and two further animals with the highest concentrations. After 24 hours, the dressing was removed and the skin cleaned of residual test substance.

If possible, the treated skin areas were assessed for irritation 24 and 48 hours after exposure.

<sup>†</sup> Supplier: Lohmann GmbH, Neuwied, Germany  
<sup>†</sup> Supplier: 3M, St. Paul, U.S.A.

MAIN STUDY

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## INDUCTION - Experimental animals

- Day 1 The scapular region was clipped and three pairs of intradermal injections (0.1 ml/site) were made in this area as follows:
- A) A 1:1 w/w mixture of Freund's Complete Adjuvant (Difco, Detroit, U.S.A.) with water for injection (Fresenius AG, Bad Homburg, Germany).
  - B) The test substance at a 10% concentration.
  - C) A 1:1 w/w mixture of the test substance, at twice the concentration used in (B) and Freund's Complete Adjuvant.

Note: One of each pair was on each side of the midline and from cranial A) to caudal C).

- Day 3 The dermal reactions caused by the intradermal injections were assessed for irritation.

- Day 8 The scapular area between the injection sites was clipped and subsequently treated with 0.5 ml of the undiluted test substance using a Metalline patch (2x3 cm) mounted on Medical tape, which was held in place with Micropore tape and subsequently Coban elastic bandage.

The dressing was removed after 48 hours exposure, the skin cleaned of residual test substance and the dermal reactions caused by the epidermal exposure were assessed for irritation.

## INDUCTION - Control animals

The control animals were treated as described for the experimental animals, except that, instead of the test substance, the vehicle was administered.

## CHALLENGE - Control and experimental animals

- Day 21 One flank of the animals was clipped and treated by epidermal application of a 20% test substance concentration and the vehicle (0.15 ml each), using Patch Test Plasters (Leukotest®, Beiersdorf Medical, Almere, The Netherlands). The patches were held in place with Micropore tape and subsequently Coban elastic bandage.

The dressing was removed after 24 hours exposure and the skin cleaned of residual test substance and vehicle. The treated sites were assessed for challenge reactions 24 and 48 hours after removal of the dressing.

OBSERVATIONS

Mortality/Viability      Twice daily

Toxicity                    At least once daily.

Body weights              Prior to start and at termination of the study.

Necropsy                    The animal found dead was subjected to necropsy for gross macroscopic examination.

Irritation                    Skin reactions were graded according to the following numerical scoring systems. Furthermore, a description of all other (local) effects was recorded. Whenever necessary, the treated skin-areas were clipped at least 3 hours before the next skin reading to facilitate scoring.

Grading Irritation Reactions:

Erythema and eschar formation:

No erythema .....	0
Slight erythema (barely perceptible) .....	1
Well-defined erythema .....	2
Moderate erythema .....	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth) .....	4

Oedema formation:

No oedema .....	0
Slight oedema (barely perceptible) .....	1
Well-defined oedema (edges of area well-defined by definite raising) .....	2
Moderate oedema (raised approximately 1 millimeter) .....	3
Severe oedema (raised more than 1 millimeter and extending beyond the area of exposure) .....	4

\*. Intradermal reactions were assessed for erythema only or, if necrosis is present, the diameter of necrosis.

Grading Challenge Reactions:

No visible change.....	0
Discrete or patchy erythema .....	1
Moderate and confluent erythema .....	2
Moderate erythema and swelling .....	3
Intense erythema and swelling .....	4

After the end of the study all animals were killed by asphyxiation using an oxygen/carbon dioxide procedure.

INTERPRETATION

The results for the experimental animals at the challenge phase were compared with the results for the control animals. Positive skin reactions (grade 1 or more) were considered signs of sensitisation, provided that such reactions were not observed or were less persistent in the control group. A sensitisation rate (%) was calculated as follows: the number of sensitised animals as a proportion of the total number of animals in the experimental group. The results were evaluated according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC).

**RESULTS**PRELIMINARY IRRITATION STUDY

The results of the intradermal injections and epidermal exposures for the selection of suitable test substance concentrations for the main study are described in Table 1. Since the severe irritation grade 4 was only observed 24 hours after injection of the 10% concentration and a moderate irritation (grade 3) remained present after 48 hours, it was considered that this concentration caused moderate irritation.

Based on these results, the test substance concentrations selected for the Main Study were a 10% concentration for the intradermal induction and the undiluted test substance for the epidermal induction exposure. A 20% test substance concentration was selected for the challenge phase.

MAIN STUDYInduction phase

The skin effects caused by the intradermal injections and epidermal exposure during the induction phase are given in Table 2.

Challenge phase

Skin reactions of grade 1 were observed in eight experimental animals in response to the 20% test substance concentration, 24 and/or 48 hours after exposure.

A skin reaction of grade 1 was observed in one control animal in response to the 20% test substance concentration, 48 hours after exposure (see Table 3).

Toxicity / Mortality

One experimental animal was found dead on day 8. Macroscopic post-mortem examination of the animal showed dark red discolouration of the lungs and haemorrhages in the lungs and a reduction in size of the thymus. It was considered that the study outcome, based on the healthy surviving animals, was not adversely affected. No further mortality occurred and no further symptoms of systemic toxicity were observed in any of the animals of the main study.

Body Weights

Body weights and body weight gain of experimental animals remained in the same range as controls over the study period (see Table 4).

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TERT-BUTYLPEROXYPIVALATE 75%  
IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245194

### CONCLUSION

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Taking into account the occurrence and intensity of the responses and comparing these with the skin reactions seen in the control animals, it was considered that hypersensitivity to TERT-BUTYLPEROXYPIVALATE 75% IN AROMATIC FREE MINERAL SPIRIT had been induced in eight (of the nine) the experimental animals. The response seen in the control animal was considered to be a non specific skin reaction. These results indicate a sensitisation rate of 89 per cent.

Based on these results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), TERT-BUTYLPEROXYPIVALATE 75% IN AROMATIC FREE MINERAL SPIRIT should be labelled as: may cause sensitisation by skin contact (R 43).

TERT-BUTYLPEROXYPIVALATE 75%  
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TABLE 1: PRELIMINARY IRRITATION STUDY

## SKIN REACTIONS AFTER INTRADERMAL INJECTION

Animal Number	Conc. %	Time after injection			
		24 hours		48 hours	
		Erythema (grade)	Necrosis (mm)	Erythema (grade)	Necrosis (mm)
24*	100		above 10		
	50		above 10		
25	20		6		6
	10	4		3	

## SKIN REACTIONS AFTER EPIDERMAL EXPOSURE

Animal Number	Body Weight (gram)	Conc. %	Time after exposure			
			24 hours		48 hours	
			Erythema	Oedema	Erythema	Oedema
22	363	100	2 t	0	1 t	0
		50	2 t	0	1 t	0
23	342	100	2 t	0	2 t	1
		50	2 t	0	1 t	0
24*	350	20				
		10				
25	380	20	0	0	0	0
		10	0	0	0	0

\*. Based on the severity of skin reactions, animal 24 was sacrificed for humane reasons, 24 hours after injection.

t. Yellow staining of the treated skin site by the test substance.

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IN AROMATIC FREE MINERAL SPIRIT

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TABLE 2: INDUCTION READINGS

Animal Number	Intradermal Injection (DAY 3)			Epidermal Exposure (DAY 10)	
	A	B	C	D	
<b>Control</b>					
31	E2	E2	E4	Erythema 0	Oedema 0
32	E3	NA	E4	0	0
33	E3	NA	E2	0	0
34	E3	E1	E3	0	0
35	E3	NA	E1	0	0
<b>Experimental</b>					
36	E3	E3	E4	3	0
37	E3	E2	E3	3	0
38	E3	E3	E3	2	0
39	E4	E3	E3	3	0
40**	E3	N1	E2		
41	E3	E3	E3	3	0
42	E3	E2	E2	2	0
43	E4	E3	E3	2	0
44	E3	N1	E3	3	0
45	E3	N1	E3	2	0

A. 1:1 Mixture of FCA and water for injection.

B. A 10% test substance concentration (Experimental); vehicle (Control).

C. 1:1 Mixture of FCA and a 20% test substance concentration (Experimental) or vehicle (Control).

D. The undiluted test substance (Experimental); vehicle (Control).

\*\* . Animal 40 was found dead on day 8.

Skin effects intradermal injections:

NA No abnormalities

E(.) Erythema (grade)

N(.) Signs of necrosis (mm in diameter)

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TERT-BUTYLPEROXYPIVALATE 75%  
IN AROMATIC FREE MINERAL SPIRIT

NC 70X Project 245194

TABLE 3: CHALLENGE READINGS

ANIMAL NO.	CHALLENGE				COMMENTS
	DAY 23 READINGS		DAY 24 READINGS		
	20%#	Vehicle	20%	Vehicle	
Control					
31	0	0	1	0	
32	0	0	0	0	
33	0	0	0	0	
34	0	0	0	0	
35	0	0	0	0	
Experimental					
36	0	0	0	0	not sensitised
37	1	0	0	0	sensitised
38	1	0	0	0	sensitised
39	2	0	1	0	sensitised
40**					
41	1	0	1	0	sensitised
42	1	0	1	0	sensitised
43	1	0	1	0	sensitised
44	1	0	1	0	sensitised
45	1	0	1	0	sensitised

#. Test substance concentration.

\*\* . Animal 40 was found dead on day 8.

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TABLE 4: BODY WEIGHTS (grams)

GROUP / SEX	ANIMAL	DAY 1	DAY 24
GROUP 1 / FEMALES (CONTROL)	31	340	450
	32	313	404
	33	345	420
	34	364	513
	35	332	450
	MEAN	339	447
	ST. DEV.	19	42
	N	5	5
GROUP 2 / FEMALES (EXPERIMENTAL)	36	358	458
	37	331	454
	38	345	485
	39	327	424
	40	331	---
	41	335	451
	42	324	455
	43	307	412
	44	356	497
	45	323	428
	MEAN	334	452
ST. DEV.	16	28	
	N	10	9

--- Animal 40 was found dead on day 8.

**B.07**

TERT-BUTYLPEROXYPIVALATE 75%  
IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245194

## APPENDIX 1

ASSESSMENT OF CONTACT HYPERSENSITIVITY TO  
ALPHA-HEXYLCINNAMIC ALDEHYDE, TECH. 85%  
IN THE ALBINO GUINEA PIG (MAXIMISATION-TEST),

a Reliability Check.

NOTOX Project 244564

## SUMMARY

A reliability check is carried out at regular intervals to check the sensitivity of the test system and the reliability of the experimental techniques as used by NOTOX. In this study, performed in August/September 1998, females of the albino Dunkin Hartley guinea pig (from Charles River, Germany) were checked for the sensitivity to ALPHA-HEXYLCINNAMICALDEHYDE, TECH. 85%. The females were approx. 5 weeks old (individual body weights <500 grams) at commencement of the study. The study was based on the OECD Guideline No. 406, the EEC Directive 92/69/EEC, Part B.6 and on the method described in 'Allergic Contact Dermatitis in the Guinea-Pig: Identification of Contact Allergens' Magnusson and Kligman, 1970. ALPHA-HEXYLCINNAMICALDEHYDE, TECH. 85% (CAS no. 101-86-0) was fabricated under lot no. 80281 and the purity was 99.4% (glc) (Aldrich Chemicals Co., Germany).

Test substance concentrations selected for this study were:  
Intradermal induction: A 5% solution in water (Milli-U, w/w).  
Epidermal induction: undiluted.  
Challenge: a 10% solution in water (w/w).

## SKIN REACTIONS IN THE CHALLENGE PHASE (Number of animals with skin reactions)

	ALPHA-HEXYLCINNAMICALDEHYDE Concentration	
	10%	vehicle
	24/48*	24/48*
Experimental group (9 females) <sup>c</sup>		
Score 3	1/2a	0/0
Score 2	8/6b	0/0
Score 1	0/1	0/0
No reactions	0/0	9/9
	a. All three animals showed scaliness. b. Three of the animals showed scaliness. c. One animal was removed from the study after showing signs of ill health.	
Control group (5 females)		
Score 1	1/0	0/0
No reactions	4/5	5/5

\*. time (hours) after the challenge exposure.

## CONCLUSION

The skin reactions in the experimental animals observed in response to the 10% test substance concentration in the challenge phase were considered indicative of sensitisation, taking into account the intensity and persistence of the response in the control animals.

These results lead to a sensitisation rate of 100 per cent to the 10% concentration. From these results, it was concluded that the female guinea pig of the albino Dunkin Hartley strain is an appropriate animal model for the performance of studies designed to evaluate the sensitising potential of a substance in a Maximisation type of test.

The raw data, protocol and report from this study are kept in the NOTOX archives. The test described above was performed in accordance with NOTOX Standard Operating Procedures and the report was audited by the QA-unit.

**B.09**

TERT-BUTYLPEROXYPIVALATE 75%  
IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245194

## APPENDIX 2

TEST SUBSTANCE CERTIFICATE OF ANALYSIS

B 10

TERT-BUTYLPEROXYPIVALATE 75%  
IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245194

24.FEV.1999 15:58

HYL LOGISTICS GELIN

15:58

P.2/3



# Certificate of analysis

Chemicals

Delivery address

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HAMBAKENWETERING 3  
5231 DD DEN BOSCH  
NETHERLANDS

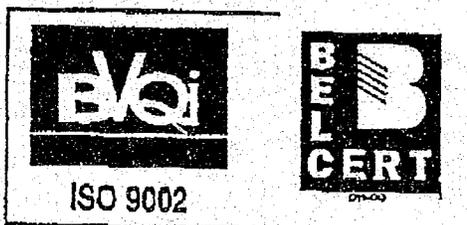
Despatch: 30/Sep/98  
ICS 331 25399 / 9807135512

Page: 1  
Chlin, date: 01/Jul/98

Order number Akzo Nobel: 6137000596		Your order number:		
Product code: 65811				
Product name: TERT-BUTYL PEROXYPIVALATE				
TRIGONOX 25 C 75				
PRODUIT FINI				
Quantity : 0.25 KG				
Packages : 01 sample x 0.25 kg.				
Batch/lot : 0419807135512				
Analysis of	Unit	Results	Specification	Test Method
COLOUR.....	Pt-Co	5	≤ 20	Col/84.3
ASSAY.....	%	74.8	74.0-76.0	Jo/72.13
TEHP content.....	mg/kg	65	≤ 1000	Gc/79.2
Inorg. and Org. Hydr. Cl.	mg/kg	1	≤ 100	Ag/90.1
For method of analysis an equivalent test method may have been used.				

Quality Control Department

Ch Lehu



S.A. Akzo Nobel Chemicals N.V.  
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V.A.T. : BE 415.916.895

B.11

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REPORT

ASSESSMENT OF CONTACT HYPERSENSITIVITY TO  
TERT-BUTYLPEROXYACETATE 50% IN AROMATIC FREE MINERAL SPIRIT  
IN THE ALBINO GUINEA PIG  
(MAXIMISATION-TEST)

NOTOX Project 245172  
NOTOX Substance 84285

- Page 1 of 20 -

*tert-Butylperoxyacetate*  
*CAS# 107-741*

B.12

TERT-BUTYLPEROXYACETATE  
50% IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245172

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CONFIDENTIALITY STATEMENT

This report contains the unpublished results of research sponsored by Akzo Nobel Chemicals B.V.. Reproduction, issue or disclosure to third parties in any form is not permitted without prior written authorization from the sponsor.

TERT-BUTYLPEROXYACETATE  
50% IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245172

STATEMENT OF GLP COMPLIANCE

---

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

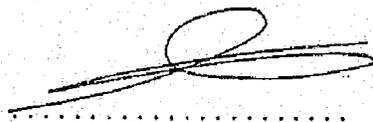
The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

Japanese Ministry of Agriculture, Forestry and Fisheries (59 NohSan, Notifications No. 3850).

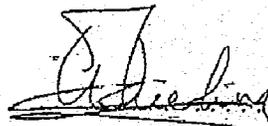
Japanese Ministry of International Trade and Industry (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85).

Study Director:  
Drs. A.H.B.M. van Huygevoort

Management:  
Drs. W.J.A.M. Frieling



Date: 26 February 1999



Date: 26 February 1999

QUALITY ASSURANCE STATEMENT

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NOTOX B.V., 's-Hertogenbosch, The Netherlands

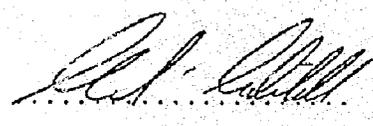
This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below. During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/ AUDITS	REPORTING DATES
on-site inspection(s)	
20 October 1998	20 October 1998
18 November 1998	18 November 1998
protocol inspection(s)	
9 October 1998	9 October 1998
report audit(s)	
13 January 1999	13 January 1999

Head of Quality Assurance:

C.J. Mitchell B.Sc.



Date: 4-3-99

## SUMMARY

Assessment for Contact Hypersensitivity to TERT-BUTYLPEROXYACETATE 50% IN AROMATIC FREE MINERAL SPIRIT in the Albino Guinea Pig (Maximisation Test).

The study was carried out based on the guidelines described in: EC Commission Directive 96/54/EC, Part B.6, 'Skin Sensitisation' and OECD No. 406, 'Skin Sensitisation', and based on the method described by Magnusson and Kligman, 'Allergic Contact Dermatitis in the Guinea Pig - Identification of Contact Allergens'.

Test substance concentrations selected for the Main study were based on the results of a preliminary study.

In the Main study, ten experimental animals were intradermally injected with a 10% concentration and epidermally exposed to a 50% concentration. Five control animals were similarly treated, but with the vehicle (Corn oil) only. Two weeks after the epidermal application all animals were challenged with a 10% test substance concentration and the vehicle.

Skin reactions of grade 1 were observed in eight experimental animals in response to the 10% test substance concentration. No skin reactions were evident in the control animals.

Scalliness was seen in the treated skin site of one experimental animal.

The skin reactions observed in response to a 10% test substance concentration in eight (of the ten) experimental animals in the challenge phase were considered indicative of sensitisation, based on the absence of any response in the control animals.

These results indicate a sensitisation rate of 80 per cent.

Based on these results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), TERT-BUTYLPEROXYACETATE 50% IN AROMATIC FREE MINERAL SPIRIT should be labelled as: may cause sensitisation by skin contact (R 43).

TERT-BUTYLPEROXYACETATE  
50% IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245172

PREFACE

---

Sponsors	Elf-Atochem S.A. Cours Michelet La Défense 10 F-92091 PARIS LA DÉFENSE CEDEX France
	Peroxid-Chemie GmbH Dr.-Gustav-Adolph-Strasse 3 D-82049 PULLACH Germany
	AKZO Nobel Chemicals B.V. P.O. Box 247 3800 AE AMERSFOORT The Netherlands
Study Monitor	Mrs. Ir. W. M. Clous AKZO Nobel Chemicals B.V.
Testing Facility	NOTOX B.V. Hambakenwetering 3 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Drs. A.H.B.M. van Huygevoort
Study Plan	Start : 19 October 1998 End : 20 November 1998

TERT-BUTYLPEROXYACETATE  
50% IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245172

TEST SUBSTANCE

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The sponsor is responsible for the completeness and GLP Compliance of all test substance data.

Identification	Tert-butylperoxyacetate 50% in aromatic free mineral spirit
Description	Clear colourless liquid
Akzo Nobel Trade Name	Trigonox F-C50
Chemical Name	Tert-butylperoxyacetate 50% in aromatic free mineral spirit
Cas-No.	107-71-1
Batch	0419805130361
Purity	See Certificate of Analysis
Test substance storage	In refrigerator in the dark Do not heat test substance
Stability under storage conditions	Not indicated
Expiry date	01 October 1999 (allocated by NOTOX, 1 year after receipt of the test substance)
Density	820 kg/m <sup>3</sup> (20°C)
Stability in vehicle	Corn oil: at least 96 h
Vehicle	Corn oil
Rationale	The vehicle was selected based on a pretest performed at NOTOX.

The test substance was not heated above the temperature of 70° C.

Preparation When required, the test substance formulations (w/w) were prepared prior to each treatment. No adjustment was made for specific gravity of vehicle. Homogeneity was obtained to visually acceptable levels.

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PURPOSE AND RATIONALE

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The purpose of this study was to evaluate whether TERT-BUTYLPEROXYACETATE 50% IN AROMATIC FREE MINERAL SPIRIT induces contact hypersensitivity in guinea pigs after intradermal and epidermal exposure of the animals under the conditions described in this report.

This study should provide a rational basis for risk assessment in man. The Maximisation test is selected because it is regarded as the most sensitive and the preferred method with regard to testing for sensitisation potential.

## GUIDELINES

The study procedures described in this report were based on the following guidelines and test method:

European Community (EC), Council Directive 67/548/EEC, as last amended by Commission Directive 96/54/EC, Annex V, Part B, Methods for the Determination of Toxicity, B.6: "Skin sensitisation", Official Journal of the European Communities No. L 248, 1996.

Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No.406, "Skin Sensitisation", Paris Cedex, 1992.

"Allergic Contact Dermatitis in the Guinea-Pig: Identification of Contact Allergens" Magnusson B. Kligman A.M., 1970 published by C.C. Thomas, Springfield, Illinois, USA.

## ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years:  
raw data, protocol, report and test substance reference sample.

## TEST SYSTEM

Species	Dunkin Hartley strain, albino guinea pig (SPF-quality) Recognised by international guidelines as the recommended test system (e.g. OECD, EC). Source : Charles River, Germany.
Number of animals	Experimental group : 10 females. Control group : 5 females. (females were nulliparous and non-pregnant).
Age and body weight	Young adult animals (approx. 4 weeks old) were selected. Individual body weights did not exceed 500 grams.
Identification	Ear tattoo.
Reliability check	The results of a reliability test performed not more than 6 months previously are summarised in the Appendix. Similar procedures were used in the reliability test and in this study.

## ANIMAL HUSBANDRY

## Conditions

Air-conditioned room with approximately 15 air changes per hour and the environment controlled with optimal conditions considered as being a temperature of 21°C and a relative humidity of 50%. Fluctuations from these optimal conditions were noted, but were considered not to have affected study integrity. Lighting was 12 hours artificial fluorescent light and 12 hours dark per day.

## Accommodation

Group housing of 5 animals per labelled metal cage with wire-mesh floors and equipped with an automatic drinking system (ITL, Bergen, The Netherlands). The acclimatisation period was at least 5 days before the start of treatment under laboratory conditions.

**Diet**

Free access to standard guinea pig diet, including ascorbic acid (1600 mg/kg); LC 23-B, pellet diameter 4mm (Hope farms, Woerden, The Netherlands). Certificates of analysis were examined and retained in the NOTOX archives. In addition, hay (B.M.I., Helmond, The Netherlands) was provided once a week.

**Water**

Free access to tap-water, diluted with decalcified water. Certificates of quarterly analysis for tap-water were examined and retained in the NOTOX archives.

**PRELIMINARY IRRITATION STUDY**

A preliminary irritation study was conducted in order to select test substance concentrations to be used in the Main Study. The selection of concentrations was based on the following criteria:

- The concentrations are well-tolerated by the animals.
- For the induction exposures: the highest possible concentration that produced mild to moderate irritation (grades 2 - 3).
- For challenge exposure: the maximum non-irritant concentration.

The test substance concentrations used were from the series:

Undiluted (if a liquid), 50%, 20%, 10%, 5%, 2%, 1% and, if needed, further lower concentrations using the same steps.

The test system, procedures and techniques were identical to those used during the main study, unless otherwise specified.

The animals were selected from stock and were between 5 and 9 weeks old, and as a consequence the body weights could exceed 500 grams. Body weights were determined prior to treatment.

**Intradermal injections:**

A series of four test substance concentrations was used; the highest concentration being the maximum concentration that could technically be injected. Each of two animals received two different concentrations in duplicate (0.1 ml/site) in the clipped scapular region. The injection sites were assessed for irritation 24 and 48 hours after treatment.

**Epidermal application:**

A series of four test substance concentrations was used; the highest concentration being the maximum concentration that could technically be applied. Two different concentrations were applied (0.5 ml each) per animal to the clipped flank, using Metalline patches<sup>†</sup> (2x3 cm) mounted on Medical tape<sup>‡</sup>, which were held in place with Micropore tape<sup>‡</sup> and subsequently Coban elastic bandage<sup>‡</sup>. The animals receiving intradermal injections were treated with the lowest concentrations and two further animals with the highest concentrations. After 24 hours, the dressing was removed and the skin cleaned of residual test substance.

The treated skin areas were assessed for irritation 24 and 48 hours after exposure.

<sup>†</sup>. Supplier: Lohmann GmbH, Neuwied, Germany

<sup>‡</sup>. Supplier: 3M, St. Paul, U.S.A.

## MAIN STUDY

## INDUCTION - Experimental animals

- Day 1 The scapular region was clipped and three pairs of intradermal injections (0.1 ml/site) were made in this area as follows:
- A) A 1:1 w/w mixture of Freund's Complete Adjuvant (Difco, Detroit, U.S.A.) with water for injection (Fresenius AG, Bad Homburg, Germany).
  - B) The test substance at a 10% concentration.
  - C) A 1:1 w/w mixture of the test substance, at twice the concentration used in (B) and Freund's Complete Adjuvant.

Note: One of each pair was on each side of the midline and from cranial A) to caudal C).

- Day 3 The dermal reactions caused by the intradermal injections were assessed for irritation.

- Day 8 The scapular area between the injection sites was clipped and subsequently treated with 0.5 ml of a 50% test substance concentration using a Metalline patch (2x3 cm) mounted on Medical tape, which was held in place with Micropore tape and subsequently Coban elastic bandage.

The dressing was removed after 48 hours exposure, the skin cleaned of residual test substance and the dermal reactions caused by the epidermal exposure were assessed for irritation.

## INDUCTION - Control animals

The control animals were treated as described for the experimental animals, except that, instead of the test substance, the vehicle was administered.

## CHALLENGE - All animals

- Day 22 One flank of all animals was clipped and treated by epidermal application of a 10% test substance concentration and the vehicle (0.15 ml each), using Patch Test Plasters (Leukotest<sup>®</sup>, Beiersdorf Medical, Almere, The Netherlands). The patches were held in place with Micropore tape and subsequently Coban elastic bandage.

The dressing was removed after 24 hours exposure and the skin cleaned of residual test substance and vehicle. The treated sites were assessed for challenge reactions 24 and 48 hours after removal of the dressing.

## OBSERVATIONS

Mortality/Viability      Twice daily

Toxicity                    At least once daily.

Body weights              Prior to start and at termination of the study.

Irritation                  Skin reactions were graded according to the following numerical scoring systems. Furthermore, a description of all other (local) effects was recorded. Whenever necessary, the treated skin-areas were clipped at least 3 hours before the next skin reading to facilitate scoring.

## Grading Irritation Reactions:

Erythema and eschar formation:

No erythema .....	0
Slight erythema (barely perceptible) .....	1
Well-defined erythema .....	2
Moderate erythema .....	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth) .....	4

Oedema formation:

No oedema .....	0
Slight oedema (barely perceptible) .....	1
Well-defined oedema (edges of area well-defined by definite raising) .....	2
Moderate oedema (raised approximately 1 millimeter) .....	3
Severe oedema (raised more than 1 millimeter and extending beyond the area of exposure) ..	4

\*. Intradermal reactions were assessed for erythema only or, if necrosis is present, the diameter of necrosis.

## Grading Challenge Reactions:

No visible change .....	0
Discrete or patchy erythema .....	1
Moderate and confluent erythema .....	2
Moderate erythema and swelling .....	3
Intense erythema and swelling .....	4

After the end of the study all animals were killed by asphyxiation using an oxygen/carbon dioxide procedure.

## INTERPRETATION

The results for the experimental animals at the challenge phase were compared with the results for the control animals. Positive skin reactions (grade 1 or more) were considered signs of sensitisation, provided that such reactions were not observed or were less persistent in the control group. A sensitisation rate (%) was calculated as follows: the number of sensitised animals as a proportion of the total number of animals in the experimental group. The results were evaluated according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC).

## RESULTS

PRELIMINARY IRRITATION STUDY

The results of the intradermal injections and epidermal exposures for the selection of suitable test substance concentrations for the main study are described in Table 1. Since the severe irritation grade 4 was only observed 24 hours after injection of the 10% concentration and only a mild irritation (grade 2) remained present after 48 hours, it was considered that this concentration caused moderate irritation.

Based on these results, the test substance concentrations selected for the Main Study were a 10% concentration for the intradermal induction and a 50% concentration for the epidermal induction exposure. A 10% test substance concentration was selected for the challenge phase.

MAIN STUDY

## Induction phase

The skin effects caused by the intradermal injections and epidermal exposure during the induction phase are given in Table 2.

## Challenge phase

Skin reactions of grade 1 were observed in eight experimental animals in response to the 10% test substance concentration. No skin reactions were evident in the control animals (see Table 3). Scaliness was seen in the treated skin site of one experimental animal.

## Toxicity / Mortality

No mortality occurred and no symptoms of systemic toxicity were observed in the animals of the main study.

## Body Weights

Body weights and body weight gain of experimental animals remained in the same range as controls over the study period (see Table 4).

CONCLUSION

The skin reactions observed in response to a 10% test substance concentration in eight (of the ten) experimental animals in the challenge phase were considered indicative of sensitisation, based on the absence of any response in the control animals. These results indicate a sensitisation rate of 80 per cent.

Based on these results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), TERT-BUTYLPEROXYACETATE 50% IN AROMATIC FREE MINERAL SPIRIT should be labelled as: may cause sensitisation by skin contact (R 43).

TERT-BUTYLPEROXYACETATE  
50% IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245172

TABLE 1: PRELIMINARY IRRITATION STUDY

SKIN REACTIONS AFTER INTRADERMAL INJECTION

Animal Number	Conc. %	Time after injection			
		24 hours		48 hours	
		Erythema (grade)	Necrosis (mm)	Erythema (grade)	Necrosis (mm)
12	100		above 10		above 10
	50		10		10
13	20		2		2
	10	4		2	

SKIN REACTIONS AFTER EPIDERMAL EXPOSURE

Animal Number	Body Weight (gram)	Conc. %	Time after exposure			
			24 hours		48 hours	
			Erythema	Oedema	Erythema	Oedema
9	487	100	4 n	2	4 n	1
		50	3	0	2	0
11	495	100	4 n	1	4 n	0
		50	1	0	1 a	0
12	442	20	1	0	0	0
		10	0	0	0	0
13	493	20	0	0	0	0
		10	0	0	0	0

n. Signs of necrosis.

a. Score given for the edges of the application area.

Note: It was noted that the identification of the animals used in the preliminary irritation test was identical to some animals in the main study. The raw data of the study specifies that they were of separate deliveries from the supplier.

TERT-BUTYLPEROXYACETATE  
50% IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245172

TABLE 2: INDUCTION READINGS

Animal Number	Intradermal Injection (DAY 3)			Epidermal Exposure (DAY 10)	
	A	B	C	D	
Control				Erythema	Oedema
1	E2	E2	E3	0	0
2	E3	E1	E3	0	0
3	E3	NA	E2	0	0
4	E3	NA	E3	0	0
5	E4	NA	E3	0	0
Experimental					
6	E2	E1	E2	2	0
7	E3	E3	E3	2	0
8	E2	NA	E3	0	0
9	E2	E1	E4	2	0
10	E3	E2	E4	2	0
11	E3	E1	E4	2	0
12	E3	E1	E3	2	0
13	E3	E1	E3	3	0
14	E2	E1	E3	1	0
15	E2	E1	E2	2	0

- A. 1:1 Mixture of FCA and water for injection.  
 B. A 10% test substance concentration (Experimental); vehicle (Control).  
 C. 1:1 Mixture of FCA and a 20% concentration (Experimental) or vehicle (Control).  
 D. A 50% test substance concentration (Experimental); vehicle (Control).

Skin effects intradermal injections:

NA No abnormalities

E(.) Erythema (grade)

N(.) Signs of necrosis (mm in diameter)

TERT-BUTYLPEROXYACETATE  
50% IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245172

TABLE 3: CHALLENGE READINGS

ANIMAL NO.	CHALLENGE				COMMENTS
	DAY 24 READINGS		DAY 25 READINGS		
	10%#	Vehicle	10%	Vehicle	
Control					
1	0	0	0	0	
2	0	0	0	0	
3	0	0	0	0	
4	0	0	0	0	
5	0	0	0	0	
Experimental					
6	1	0	0	0	sensitised
7	1	0	1	0	sensitised
8	1	0	1	0	sensitised
9	1	0	1 p	0	sensitised
10	1	0	1	0	sensitised
11	1	0	1	0	sensitised
12	1	0	1	0	sensitised
13	0	0	1	0	sensitised
14	0	0	0	0	not sensitised
15	0	0	0	0	not sensitised

#. Test substance concentration.

p. Scaliness.

TERT-BUTYLPEROXYACETATE  
50% IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245172

TABLE 4: BODY WEIGHTS (grams)

GROUP / SEX	ANIMAL	DAY 1	DAY 25
GROUP 1 / FEMALES (CONTROL)	1	319	427
	2	326	471
	3	338	451
	4	369	555
	5	346	468
	MEAN	340	474
	ST. DEV.	19	48
	N	5	5
GROUP 2 / FEMALES (EXPERIMENTAL)	6	346	495
	7	329	465
	8	316	414
	9	315	427
	10	318	385
	11	339	464
	12	313	432
	13	359	490
	14	303	430
	15	332	494
	MEAN	327	450
	ST. DEV.	17	38
	N	10	10

C.13

TERT-BUTYLPEROXYACETATE  
50% IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245172

## APPENDIX 1

ASSESSMENT OF CONTACT HYPERSENSITIVITY TO  
ALPHA-HEXYLCINNAMIC ALDEHYDE, TECH. 85%  
IN THE ALBINO GUINEA PIG (MAXIMISATION-TEST),

a Reliability Check.

NOTOX Project 244564

## SUMMARY

A reliability check is carried out at regular intervals to check the sensitivity of the test system and the reliability of the experimental techniques as used by NOTOX. In this study, performed in August/September 1998, females of the albino Dunkin Hartley guinea pig (from Charles River, Germany) were checked for the sensitivity to ALPHA-HEXYLCINNAMICALDEHYDE, TECH. 85%. The females were approx. 5 weeks old (individual body weights <500 grams) at commencement of the study. The study was based on the OECD Guideline No. 406, the EEC Directive 92/69/EEC, Part B.6 and on the method described in 'Allergic Contact Dermatitis in the Guinea-Pig: Identification of Contact Allergens' Magnusson and Kligman, 1970. ALPHA-HEXYLCINNAMICALDEHYDE, TECH. 85% (CAS no. 101-86-0) was fabricated under lot no. 80281 and the purity was 99.4% (glc) (Aldrich Chemicals Co., Germany).

Test substance concentrations selected for this study were:  
Intradermal induction: A 5% solution in water (Milli-U, w/w).  
Epidermal induction: undiluted.  
Challenge: a 10% solution in water (w/w).

## SKIN REACTIONS IN THE CHALLENGE PHASE (Number of animals with skin reactions)

	ALPHA-HEXYLCINNAMICALDEHYDE Concentration	
	10%	vehicle
	24/48*	24/48*
Experimental group (9 females) <sup>c</sup>		
Score 3	1/2a	0/0
Score 2	8/6b	0/0
Score 1	0/1	0/0
No reactions	0/0	9/9
	a. All three animals showed scaliness. b. Three of the animals showed scaliness. c. One animal was removed from the study after showing signs of ill health.	
Control group (5 females)		
Score 1	1/0	0/0
No reactions	4/5	5/5

\*. time (hours) after the challenge exposure.

## CONCLUSION

The skin reactions in the experimental animals observed in response to the 10% test substance concentration in the challenge phase were considered indicative of sensitisation, taking into account the intensity and persistence of the response in the control animals.

These results lead to a sensitisation rate of 100 per cent to the 10% concentration. From these results, it was concluded that the female guinea pig of the albino Dunkin Hartley strain is an appropriate animal model for the performance of studies designed to evaluate the sensitising potential of a substance in a Maximisation type of test.

The raw data, protocol and report from this study are kept in the NOTOX archives. The test described above was performed in accordance with NOTOX Standard Operating Procedures and the report was audited by the QA-unit.

**D.01**

TERT-BUTYLPEROXYACETATE  
50% IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245172

## APPENDIX 2

TEST SUBSTANCE CERTIFICATE OF ANALYSIS

D 02

TERT-BUTYLPEROXYACETATE  
50% IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245172

29.FEV.1999 15:53 PNC LOGISTICS GHIN

NOTOX P.4/5



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NETHERLANDS

Despatch: 30/Sep/98  
ICS 331 25399 / 9805130361

Page: 1  
Ghln. date: 15/May/98

Order number Akzo Nobel: 6137000596 Your order number:  
Product code: 65861  
Product name: TERT-BUTYL PEROXYACETATE  
TRIGONOX FC 50  
PRODUIT FINI

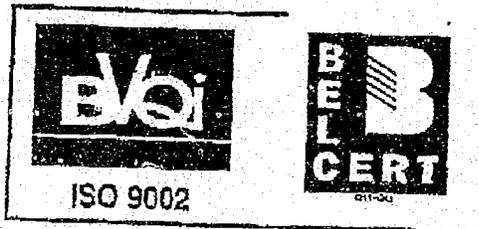
Quantity : 0.25 KG  
Packages : 01 sample x 0.25 kg.  
Batch/lot : 0419805130361

Analysis of	Unit	Results	Specification	Test Method
Colour.....	Pt-Co	5	≤ 20	Col/94.3
ASSAY.....	%	50,6	4) 0-51.0	Jo/72.13
TBHP content.....	ug/kg	49	≤ 1000	Pot/94.2
Inorg. and Org. Hydr. Cl.	mg/kg	1	≤ 50	Ag/90.1

For method of analysis an equivalent test method may have been used.

Quality Control Department

Ch Lehu



S.A. Akzo Nobel Chemicals N.V.  
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Zone A  
B-7011 Ghlin, Belgique  
Tél. : +32 (0)65 64 23 81  
64 23 28  
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Reg. Comm. : Mons 93.127  
V.A.T. : BE 415.916.895

Product manufactured and analysed at an ISO 9002 - certified facility

D. 03

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1999 SEP -3 AM 7:18

REPORT

PRIMARY SKIN IRRITATION/CORROSION STUDY WITH

TERT-BUTYLPEROXY ISOPROPYL CARBONATE, (CAS#2372-21-6)  
75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT

IN THE RABBIT

(4-HOUR SEMI-OCCLUSIVE APPLICATION)

NOTOX Project 245205  
NOTOX Substance 84303

D. 04

TERT-BUTYLPEROXY ISOPROPYL CARBONATE,  
75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245205

---

CONFIDENTIALITY STATEMENT

This report contains the unpublished results of research sponsored by Akzo Nobel Chemicals B.V.. Reproduction, issue or disclosure to third parties in any form is not permitted without prior written authorization from the sponsor.

**D. 05.**

TERT-BUTYLPEROXY ISOPROPYL CARBONATE,  
75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245205

STATEMENT OF GLP COMPLIANCE

---

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

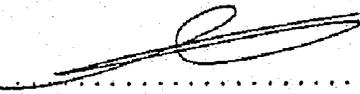
The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

Japanese Ministry of Agriculture, Forestry and Fisheries (59 NohSan, Notifications No. 3850).

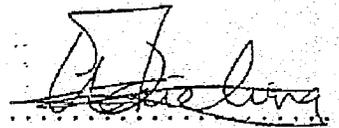
Japanese Ministry of International Trade and Industry (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85).

Study Director:  
Drs. A.H.B.M. van Huygevoort

Management:  
Drs. W.J.A.M. Frieling



Date: 26 February 1999



Date: 26 February 1999

D.06

TERT-BUTYLPEROXY ISOPROPYL CARBONATE,  
75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245205

QUALITY ASSURANCE STATEMENT

---

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below. During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/ AUDITS	REPORTING DATES
on-site inspection(s)	
13 October 1998	13 October 1998
protocol inspection(s)	
9 October 1998	9 October 1998
report audit(s)	
26 November 1998	26 November 1998

Head of Quality Assurance:

C.J. Mitchell B.Sc.

.4.3.99.....  
Date: *C.J. Mitchell*

TERT-BUTYLPEROXY ISOPROPYL CARBONATE,  
75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245205

SUMMARY

---

Primary skin irritation/corrosion study with TERT-BUTYLPEROXY ISOPROPYL CARBONATE, 75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT in the rabbit (4-hour semi-occlusive application).

The study was carried out based on the guidelines described in: EC Commission Directive 92/69/EEC, B.4, "Acute Toxicity - Skin irritation" and OECD No.404, "Acute Dermal Irritation/Corrosion".

Three rabbits were exposed to 0.5 ml of TERT-BUTYLPEROXY ISOPROPYL CARBONATE, 75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT, applied onto clipped skin for 4 hours using a semi-occlusive dressing. Observations were made 1, 24, 48 and 72 hours and 7, 14 and 21 days after exposure.

Exposure to TERT-BUTYLPEROXY ISOPROPYL CARBONATE, 75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT resulted in moderate to severe erythema and severe oedema in the treated skin-areas of the three rabbits. In all animals, reduced flexibility of the skin, scaliness and/or bald skin were present 7 and 14 days after exposure. Bald skin remained present until termination but the skin irritation had resolved within 21 days after exposure in all animals.

No evidence of full thickness destruction of the skin or scar tissue was observed during the observation period, indicating that no corrosion of the skin had occurred by dermal application of TERT-BUTYLPEROXY ISOPROPYL CARBONATE, 75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT to the intact rabbit skin.

Based on the results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), TERT-BUTYLPEROXY ISOPROPYL CARBONATE, 75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT should be labelled as: irritating to skin (R 38).

PREFACE

---

Sponsors

Elf-Atochem S.A.  
Cours Michelet La Défense 10  
F-92091 PARIS LA DÉFENSE CEDEX  
France

Peroxid-Chemie GmbH  
Dr.-Gustav-Adolph-Strasse 3  
D-82049 PULLACH  
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AKZO Nobel Chemicals B.V.  
P.O. Box 247  
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Study Monitor

Mrs. Ir. W. M. Clous  
AKZO Nobel Chemicals B.V.

Testing Facility

NOTOX B.V.  
Hambakenwetering 3  
5231 DD 's-Hertogenbosch  
The Netherlands

Study Director

Drs. A.H.B.M. van Huygevoort

Study Plan

Start : 20 October 1998  
End : 10 November 1998

# D. 09

TERT-BUTYLPEROXY ISOPROPYL CARBONATE,  
75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245205

## TEST SUBSTANCE

---

The sponsor is responsible for the completeness and GLP Compliance of all test substance data.

Identification	Tert-butylperoxy isopropyl carbonate, 75% solution in aromatic free mineral spirit
Description	Clear colourless liquid
Akzo Nobel Trade Name	Trigonox BPIC-C75
Chemical Name	Tert-butylperoxy isopropyl carbonate, 75% solution in aromatic free mineral spirit
Cas-No.	2372-21-6
Batch	0419804130350
Purity	See Certificate of Analysis
Test substance storage	In refrigerator in the dark Do not heat test substance
Stability under storage conditions	Not indicated
Expiry date	01 October 1999 (allocated by NOTOX, 1 year after receipt of the test substance)
Density	900-910 kg/m <sup>3</sup> (20°C)

The test substance was not heated above the temperature of 70°C.

Preparation                      The test substance was applied undiluted as delivered by the sponsor.

## PURPOSE AND RATIONALE

---

The purpose of this primary skin irritation study was to assess the possible irritation or corrosion potential of a single dose of TERT-BUTYLPEROXY ISOPROPYL CARBONATE, 75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT when placed on the skin of rabbits.

This study should provide a rational basis for risk assessment in man. The absence of skin pigmentation in the albino rabbit facilitates the evaluation of induced skin reactions. The dermal route was selected because TERT-BUTYLPEROXY ISOPROPYL CARBONATE, 75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT may accidentally come into contact with the skin during manufacture, handling and/or use.

GUIDELINES

---

The study procedures described in this report were based on the following guidelines:

European Community (EC), Council Directive 67/548/EEC, as last amended by Commission Directive 92/69/EEC, Annex V, Part B, Methods for the Determination of Toxicity, B.4: 'Acute Toxicity - Skin Irritation'. Official Journal of the European Communities No. L 383, 1992

Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No. 404: 'Acute Dermal Irritation / Corrosion', Paris Cedex, 1992.

ARCHIVING

---

NOTOX B.V. will archive the following data for at least 10 years:  
raw data, protocol, report and test substance reference sample.

TEST SYSTEM

---

Species	Albino Rabbit, New Zealand White, (SPF-Quality) Recognised by international guidelines as the recommended test system (e.g. EC, OECD) Source: Broekman Institute, Someren, The Netherlands.
Number of animals	3 Animals of either sex.
Age and body weight	Animals used within the study were at least 6 weeks old and body weights were less than 3.5 kg.
Identification	Ear tag.

ANIMAL HUSBANDRY

---

## Conditions

Air-conditioned room with approximately 15 air changes per hour and the environment controlled with optimal conditions considered as being a temperature of 21°C and a relative humidity of 50%. Fluctuations from these optimal conditions were noted, but were considered not to have affected study integrity. Lighting was 12 hours artificial fluorescent light and 12 hours dark per day.

## Accommodation

Individually housed in labelled cages with perforated floors (Scanbur, Denmark) and equipped with an automatic drinking system (ITL, Bergen, The Netherlands). Acclimatisation period was at least 5 days before start of treatment under laboratory conditions.

## Diet

Standard laboratory rabbit diet (LKK-20, pellet diameter 4mm, Hope Farms, Woerden, The Netherlands) approx. 100 gram per day. Certificates of analysis were examined and retained in the NOTOX archives.  
In addition, hay (BMI, Helmond, The Netherlands) was provided once a week.

## Water

Free access to tap-water diluted with decalcified water. Certificates of quarterly analysis were examined and retained in the NOTOX archives.

## TREATMENT

Approximately 24 hours before treatment, the dorsal fur was clipped with electric clippers, exposing an area of approximately 150 square centimeters (10x15 cm<sup>2</sup>). Whenever considered necessary the treated skin areas were re-clipped at least 3 hours before the observations, to facilitate the scoring.

A health inspection was performed prior to the commencement of treatment, to ensure that the animals were in a good state of health. Special attention was paid to the skin to be treated, which was intact and free from abnormalities.

On test day 1, 0.5 ml of the test substance was applied to the skin of one flank, using a Metalline patch<sup>#</sup> of 2x3 cm. The patch was mounted on Micropore tape, which was wrapped around the abdomen and secured with Coban elastic bandage.

Four hours after the application, the dressing was removed and the remaining test substance removed using a tissue moistened with tap-water and subsequently a dry tissue.

- <sup>#</sup>. Supplier: Lohmann GmbH, Neuwied, Germany  
 . Supplier: 3M, St. Paul, U.S.A.

## OBSERVATIONS

Mortality/Viability	Twice daily.
Toxicity	At least once daily.
Body Weight	Day of treatment (prior to application).
Irritation	The skin reactions were assessed at approximately 1, 24, 48 and 72 hours and 7, 14 and 21 days after the removal of the dressings and test substance. The irritation scores and a description of all other (local) effects were recorded. Adjacent areas of the untreated skin of each animal served as controls.

The irritation was assessed according to the following numerical scoring system. At each observation, the highest scores given was recorded:

## ERYTHEMA AND ESCHAR FORMATION

No erythema .....	0
Very slight erythema (barely perceptible) .....	1
Well defined erythema .....	2
Moderate to severe erythema .....	3
Severe erythema (beet redness) .....	4

\* In cases where signs of necrosis or corrosion (injuries in depth) prevent erythema scoring, the maximum grade for erythema (= 4) is given.

## 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 mm) .....	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure) .....	4

---

**INTERPRETATION**

---

The results were evaluated according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC).

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**RESULTS**

---

**Irritation**

Four hours exposure to 0.5 ml of TERT-BUTYLPEROXY ISOPROPYL CARBONATE, 75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT resulted in moderate to severe erythema and severe oedema in the treated skin-areas of the three rabbits. In all animals, reduced flexibility of the skin, scaliness and/or bald skin were present 7 and 14 days after exposure. Bald skin remained present until termination but the skin irritation had resolved within 21 days after exposure in all animals.

**Corrosion**

There was no evidence of a corrosive effect on the skin.

**Colouration**

No staining of the treated skin by the test substance was observed.

**Toxicity / Mortality**

No symptoms of systemic toxicity were observed in the animals during the test period and no mortality occurred.

---

**CONCLUSION**

---

No evidence of full thickness destruction of the skin or scar tissue was observed during the observation period, indicating that no corrosion of the skin had occurred by dermal application of TERT-BUTYLPEROXY ISOPROPYL CARBONATE, 75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT to the intact rabbit skin.

Based on the results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), TERT-BUTYLPEROXY ISOPROPYL CARBONATE, 75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT should be labelled as: irritating to skin (R 38).

TABLE 1

## INDIVIDUAL SKIN IRRITATION SCORES

Animal no. #	1357			1358			1361		
	Erythema	Oedema	Comments	Erythema	Oedema	Comments	Erythema	Oedema	Comments
Time after exposure									
1 hour	2	4	-	3	4	-	2	4	-
24 hours	3	3	-	3	3	-	2	3	-
48 hours	3	3	-	3	2	-	3	2	-
72 hours	3	2	-	3	2	-	3	2	-
7 days	3	2	f1	2	3	f1	2	3	f1
14 days	2	1	h1	2	1	h1	2	1	h1
21 da,	0	0	h	0	0	h	0	0	h

## Comments:

- f. Reduced flexibility of the skin.  
h. Bald skin.  
l. Scaliness.

TABLE 2

## MEAN VALUES OF SKIN IRRITATION SCORES (24, 48 and 72 h after exposure).

Animal no. #	Mean 24 - 72 hours	
	Erythema	Oedema
1357	3.0	2.7
1358	3.0	2.3
1361	2.7	2.3

## #. Animal specifications:

Animal no.	Sex	At commencement of the study	
		Age (weeks)	Body weight (grams)
1357	♂	10	1875
1358	♂	10	2025
1361	♂	10	2144

D 14

TERT-BUTYLPEROXY ISOPROPYL CARBONATE,  
75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245205

APPENDIX 1

TEST SUBSTANCE CERTIFICATE OF ANALYSIS

TERT-BUTYLPEROXY ISOPROPYL CARBONATE,  
75% SOLUTION IN AROMATIC FREE MINERAL SPIRIT

NOTOX Project 245205



# Certificate of analysis

**Chemicals**

Delivery address

NOTOX B.V.  
HAMBAKENWETERING 3  
5231 DD DEN BOSCH  
NETHERLANDS

Despatch: 30/Sep/98  
ICS 331 25399 / 9805130350

Page: 1  
Chlin, date: 31/Aug/98

Order number Akzo Nobel: 6137000596 Your order number:  
Product code: 66169  
Product name: TERT-BUTYLPERISOPROPYLCARBONATE  
TRIGONOX BPIC - C 75  
PRODUIT FINI

Quantity : 0.25 KG  
Packages : 01 sample x 0.25 kg.  
Batch/lot : 0419805130350

Analysis of	Unit	Results	Specification	Test Method
Colour.....	Pt-Co	5	≤ 10	Col/84.3
ASSAY.....	%	75.4	74.0-76.0	Jo/72.13
TBHP.....	mg/kg	741	≤ 1000	For/94.2
Energy and Org. Hydr. Chl	mg/kg	21	≤ 150	Ag/90.1

For method of analysis an equivalent test method may have been used.

Quality Control Department

*Ch Lehu*  
Ch Lehu



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Téléfax : +32 (0)65 64 23 85  
Reg. Comm. : Mons 95.427  
V.A.T. : BE 415.916.895



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SPONSOR

Elit Atochem S.A.  
Cours Michelet  
La Défense 10  
92091 Paris-la-Défense CEDEX  
France

TEST SUBSTANCE  
THIOACETIC ACID , CAS# 507-095

STUDY TITLE  
ACUTE EYE IRRITATION  
IN RABBITS

STUDY DIRECTOR  
Xavier Manciaux

STUDY COMPLETION DATE  
17 May 1999

PERFORMING LABORATORY  
CIT  
Centre International de Toxicologie  
BP 563 - 27005 Evreux - France

LABORATORY STUDY NUMBER  
17965 TAL

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APPENDICES

- 1. Test article description and analytical certificate
- 2. Diet formula

15  
16  
19 and 20

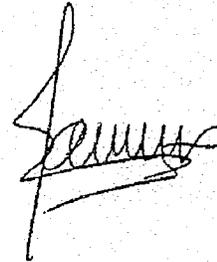
STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
- Décret N° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 9 mars 1990), Ministère de l'Industrie et de l'Aménagement du Territoire.
- Council Directive 87/18/EEC of 18 December 1986 on the harmonization of laws, regulations or administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L 15 of 17.1.87).

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT, Centre International de Toxicologie, Miserey, 27005 Evreux, France.



Toxicology

X. Manciaux  
Study Director  
Doctor of Pharmacy

Date: 17 May 1999

OTHER SCIENTISTS INVOLVED IN THIS STUDY

For Pharmacy: P.O. Guillaumat  
Doctor of Pharmacy

For Toxicology: C. Pelcot  
Study Supervisor

STATEMENT OF QUALITY ASSURANCE UNIT

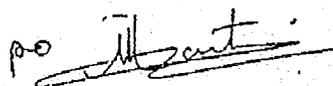
Type of inspections	Dates		
	Inspections	Reported to Study Director (*)	Reported to Management (*)
Protocol	9 December 1998	10 December 1998	10 December 1998
Report	31 March 1999	31 March 1999	12 May 1999

At about the same time as the study described in this report, "process-based" and routine facility inspections of critical procedures relevant to this study type were made by the Quality Assurance Unit.

The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and the Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.



L. Valette-Talbi Date: 17 May 1999  
Doctor of Biochemistry  
Head of Quality Assurance Unit  
and Scientific Archives

(\*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

## SUMMARY

At the request of Elf Atochem S.A., Paris-la-Défense, France, the potential of the test substance THIOACETIC ACID (batch No. AOGHJU (14/09/98)) to induce ocular irritation was evaluated in rabbits according to OECD (No. 405, 24th February 1987) and EC (92/69/EEC, B.5, 31st July 1992) guidelines.

The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

## Methods

The study design was established according to available information on the test substance and the above guidelines.

As possible irritant effects were anticipated, a first assay was conducted in one male New Zealand White rabbit.

Since the test substance showed severe irritant properties in this first assay, the study was considered complete and the test substance was not evaluated in other animals.

A single dose of 0.1 ml of the undiluted test substance was instilled into the left conjunctival sac. The right eye served as control.

The eyes were not rinsed after administration of the test substance.

Ocular reactions were observed approximately 1 hour, 24, 48 and 72 hours after the administration.

The mean values of the scores for chemosis and corneal opacity were calculated.

## Results

Slight or moderate conjunctival reactions (slight or moderate chemosis, whitish coloration of the conjunctivae and clear to whitish purulent discharge) were observed between day 1 and day 4.

A moderate corneal opacity was recorded between day 1 and day 4; the scoring of iritis was masked by this important corneal opacity.

In the view of these ocular reactions, the animal was killed on day 4 for ethical reasons.

Mean scores calculated over 24, 48 and 72 hours were 3.0 for chemosis and 3.0 for corneal opacity. Other scores were not calculable.

## Conclusion

Under our experimental conditions, the test substance THIOACETIC ACID (batch No. AOGHJU (14/09/98)) is severely irritant when administered by ocular route to rabbits.

## RESUME

A la demande de Elf Atochem S.A., Paris-la-Défense, France, les propriétés irritantes oculaires du produit THIOACETIC ACID (lot n° AOGHJU (14/09/98)) après application unique chez le Lapin ont été évaluées selon les lignes directrices de l'OCDE (n° 405, 24 février 1987) et de la CEE (92/69/EEC, B.5, 31 juillet 1992).

L'étude a été réalisée conformément aux règles de Bonnes Pratiques de Laboratoire.

## Méthode

L'étude a été réalisée selon les informations disponibles sur le produit et les lignes directrices mentionnées ci-dessus.

Des effets irritants étant supposés, un premier essai a été effectué sur 1 lapin mâle New Zealand White.

Le produit ayant montré des propriétés irritantes graves lors de ce premier essai, l'étude a été considérée complète et aucun autre animal n'a été traité.

Une dose unique de 0,1 ml de produit non dilué a été instillée dans le cul de sac conjonctival de l'oeil gauche. L'oeil droit a servi de témoin.

Aucun rinçage des yeux n'a été réalisé après l'administration du produit.

Les réactions oculaires ont été observées environ 1 heure, 24, 48 et 72 heures après l'administration.

La moyenne des scores pour le chémosis et l'opacité de la cornée a été calculée.

## Résultats

Des réactions conjonctivales légères ou modérées (chémosis léger ou modéré, coloration blanchâtre de la conjonctive et larmoiement clair à purulent blanchâtre) sont observées entre les jours 1 et 4.

Une opacité cornéenne modérée est enregistrée entre les jours 1 et 4 ; l'évaluation de l'iritis est masquée par cette importante opacité cornéenne.

Au vu de ces réactions oculaires, l'animal est sacrifié au jour 4 pour des raisons éthiques.

La moyenne des scores enregistrés après 24, 48 et 72 heures est de 3,0 pour le chémosis et 3,0 pour l'opacité cornéenne. Les autres scores ne sont pas calculables.

## Conclusion

Dans nos conditions expérimentales, le produit THIOACETIC ACID (lot n° AOGHJU (14/09/98)) est considéré sévèrement irritant par voie oculaire chez le Lapin.

## 1. INTRODUCTION

The objective of this study was to evaluate the potential of the test substance THIOACETIC ACID to induce irritation following a single ocular administration in rabbits.

In the assessment of the toxic characteristics of a test substance, determination of the irritant effects on the eyes of mammals is an important initial step. Information derived from this test serves to indicate the possible hazards likely to arise from exposure of the eyes, and associated mucous membranes, to the test substance.

This study was conducted in compliance with:

- . OECD guideline No. 405, 24th February 1987,
- . EC Directive No. 92/69/EEC, B.5, 31st July 1992.

## 2. MATERIALS AND METHODS

### 2.1 TEST SUBSTANCE

#### 2.1.1 Identification

The test substance THIOACETIC ACID used in the study was supplied by Elf Atochem S.A.

The test substance was identified as follows:

- . name:
  - protocol and labelling: THIOACETIC ACID
- . batch number:
  - protocol and labelling: AOGHJU (14/09/98)
- . Elf Atochem filing number: CAL 3871/98
- . description: colourless to pale yellow liquid
- . container: one plastic flask
- . date of receipt: 4 December 1998
- . storage conditions: at room temperature and protected from light and humidity
- . purity: 99.58%
- . expiry date: December 1999.

Data relating to the characterization of the test substance are documented in a test article description and an analytical certificate (presented in appendix 1) provided by the Sponsor.

The pH of the test substance, measured at CIT, was approximately 3.

#### 2.1.2 Formulation procedure

The test substance was used undiluted.

## 2.2 TEST SYSTEM

### 2.2.1 Animal

Sex, species, strain: male New Zealand White rabbit.

Reason for this choice: species generally accepted by regulatory authorities for this type of study.

Breeder: Elevage Cunicole de Val de Selle, 80160 Prouzel, France.

Number and identification: one animal was used. The animal was identified with a metal tag in the ear.

Weight: on the day of treatment, the animal had a body weight of 2.6 kg.

Acclimatization: at least 5 days before the beginning of the study.

### 2.2.2 Environmental conditions

The conditions in the animal room were set as follows:

. temperature:  $18 \pm 3^{\circ}\text{C}$

. relative humidity: 30 to 70%

. light/dark cycle: 12 h/12 h

. ventilation: approximately 12 cycles/hour of filtered, non-recycled air.

The temperature and relative humidity were under continuous control and recording. The records were checked daily and filed. In addition to these daily checks, the housing conditions and corresponding instrumentation and equipment were verified and calibrated at regular intervals.

The animal was housed in a polystyrene cage (48.2 cm x 58 cm x 36.5 cm) equipped with a food container and a water bottle.

### 2.2.3 Food and water

During the study, the animal had free access to 112 C pelleted diet (UAR, 91360 Villemoisson-sur-Orge, France).

Each batch of food was analysed by the supplier for composition and contaminant levels.

The diet formula is presented in appendix 2.

Drinking water filtered by a FG Millipore membrane (0.22 micron) was provided *ad libitum*.

Bacteriological and chemical analyses of the water and diet, including the detection of possible contaminants (pesticides, heavy metals and nitrosamines), are performed regularly by external laboratories.

The results of these analyses are archived at CIT.

No contaminants were known to have been present in the diet, drinking water or bedding material at levels which may be expected to have interfered with or prejudiced the outcome of the study.

## 2.3 TREATMENT

### 2.3.1 Selection of the animal

The day before treatment, the eyes of the animal were examined in order to ensure that the animal did not have signs of ocular irritation or injury or ocular defects.

### 2.3.2 Study design

The study design was established according to available information on the test substance and according to the OECD (No. 405) and EC (92/69/EEC, B.5) guidelines.

As possible irritant effects were anticipated, the test substance was evaluated in one animal (No. 600) in a first assay.

Since the test substance showed severe irritant properties in this first assay, the study was considered complete and the test substance was not evaluated in other animals.

### 2.3.3 Administration of the test substance

The test substance was used undiluted.

A single dose of 0.1 ml of the test substance was instilled into the conjunctival sac of the left eye after gently pulling the lower lid away from the eyeball.

The lower and upper eyelids were held together for about one second to avoid any loss of test substance. The right eye, which remained untreated, served as control.

The eyes were not rinsed after administration of the test substance.

### 2.3.4 Date of treatment

Animal number	Date of treatment (day 1)	End of the observation period
600	26 January 1999	29 January 1999

## 2.4 OCULAR EXAMINATIONS

The eyes were examined approximately 1 hour, 24, 48 and 72 hours after administration of the test substance.

Following the OECD and EC guidelines:

- . when there was no evidence of irritation after 72 hours, the study was ended.
- . when there was persistent ocular irritation after 72 hours, the observation period was extended to a maximum of 21 days (until day 22) in order to determine the progress of the lesions and their reversibility.
- . when severe irritant effects were observed, the animals were killed on humane grounds.

Any change in the animal behaviour was noted.

## 2.5 DESCRIPTION AND EVALUATION OF OCULAR REACTIONS

Ocular reactions were evaluated for the animal according to the following numerical scale:

### 2.5.1 Conjunctival lesions and discharge

Chemosis (lids and/or nictitating membranes)

. no swelling.....	0
. any swelling above normal (includes nictitating membranes).....	1
. obvious swelling with partial eversion of lids.....	2*
. swelling with lids about half-closed.....	3*
. swelling with lids more than half-closed.....	4*

Redness (refers to palpebral and bulbar conjunctivae, cornea and iris)

. blood vessels normal.....	0
. a number of blood vessels definitely hyperemic (injected).....	1
. diffuse, crimson colour, individual vessels not easily discernible.....	2*
. diffuse, beefy red.....	3*

Discharge

. absence of discharge.....	0
. slight discharge (does not include small amounts normally found in inner canthus).....	1
. discharge with moistening of lids and hairs adjacent to lids.....	2
. discharge with moistening of lids and hairs on wide area around the eye.....	3

### 2.5.2 Iris lesions

. normal.....	0
. markedly deepened rugae, congestion, swelling, moderate circum-corneal hyperemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive).....	1*
. no reaction to light, haemorrhage, gross destruction (any or all of these).....	2*

### 2.5.3 Corneal lesions

Cornea (direct examination or, if necessary, with an Ultra-Violet lamp)

To determine the presence or absence of corneal opacification and to evaluate the affected area, one or two drops of 0.5% sodium fluorescein solution can be instilled into the eye (however, this must not be performed before the 24-hour reading).

If corneal opacification is difficult to determine, the eye can be examined under a UV lamp (a clear fluorescence is visible in the areas of opacification).

Opacity (degree of intensity: area most dense taken for reading)

. no ulceration or opacity.....	0
. scattered or diffuse areas of opacity (other than slight dulling or normal lustre), details of iris clearly visible.....	1*
. easily discernible translucent area, details of iris slightly obscured.....	2*
. nacrous areas, no details of iris visible, size of pupil barely discernible.....	3*
. opaque cornea, iris not discernible through the opacity.....	4*

\* indicates positive effect

Area of opacity

- . one quarter (or less) but not zero ..... 1
- . greater than one quarter but less than a half ..... 2
- . greater than one half but less than three quarters ..... 3
- . greater than three quarters up to whole area ..... 4

Any other lesions observed were noted.

2.6 PROTOCOL ADHERENCE

The study was performed in accordance with Study Protocol No. 17965 TAL and subsequent amendments, with the following deviation from the agreed Study Protocol:

- . the relative humidity recorded in the animal room was sometimes outside of the target ranges specified in the protocol.

This minor deviation was not considered to compromise the validity or integrity of the study.

2.7 ARCHIVING

The study documentation and specimens generated during the course of the study are archived at CIT, 27005 Miserey, Evreux, France, for 10 years after the end of the *in vivo* phase of the study.

The archived study materials include:

- . protocol and possible amendments,
- . raw data,
- . correspondence,
- . final report and possible amendments.

On completion of this period, the archived study materials will be returned to the Sponsor, or may be archived at CIT for a further period.

### 3. RESULTS (table 1)

Slight or moderate conjunctival reactions were observed between day 1 and day 4: a slight or moderate chemosis (grade 2 or 3), a whitish coloration of the conjunctivae and a clear to whitish purulent discharge were noted.

A moderate corneal opacity (grade 3) was recorded between day 1 and day 4; the scoring of iritis was masked by this important corneal opacity.

In the view of these ocular reactions, the animal was killed on day 4 for ethical reasons.

Mean scores calculated over 24, 48 and 72 hours were 3.0 for chemosis and 3.0 for corneal opacity. Other scores were not calculable.

### 4. CONCLUSION

Under our experimental conditions, the test substance THIOACETIC ACID (batch No. AOGHJU (14/09/98)) is severely irritant when administered by ocular route to rabbits.

Table 1: Individual ocular examinations and mean values of the scores recorded at each reading (24, 48 and 72 hours)

Rabbit number	Region of eye	Description of ocular reactions	Scores				Mean irritation score (1)	Interpretation (+) (-)
			1h D1	24h D2	48h D3	72h D4		
600	Conjunctivae	Chemosis	2	3	3	3	3.0	(+)
		Redness	G	G	G	G	(2)	(2)
		Discharge	E	2	S	S	(2)	
	Iris		0	OP	OP	OP	(2)	(2)
	Corneal opacity	Intensity	3	3	3	3	3.0	(+)
		Area	3	4	4	4	4.0	
Other		*	*	*	*			
Fluorescein		/	/	/	/			

(1) mean of scores on days 2, 3 and 4

h = hour

D = day

(+) = irritant according to E.E.C. criteria

(-) = non-irritant according to E.E.C. criteria

\* = None

(2) = not calculated

/ = Fluorescein not used

E = Scoring masked by residual test substance

S = Whitish purulent discharge

OP = Scoring masked by marked corneal opacity

G = Scoring masked by a whitish colouration of the conjunctivae

APPENDICES

**1. Test article description and analytical certificate**

TOXICOLOGY DEPARTMENT  
CONFIDENTIAL  
26 November 1998

elf atochem s.a.  
La défense 10, cedex 42  
92091 Paris-la-Défense, France

## TEST ARTICLE DESCRIPTION

## THIOACETIC ACID

## STRUCTURAL FORMULA

CH<sub>3</sub>-COSH

## IDENTITY

Test article name : Thioacetic acid  
Chemical name : Ethanethioic acid  
CAS number : 507-09-5  
EINECS number : 2080638  
Molecular formula : C<sub>2</sub>H<sub>4</sub>OS  
Molecular weight : 76.11  
Purity : 99.58% (w/w)  
Origin : Elf Aquitaine Exploration Production France  
Batch : AOGHJU  
Elf Atochem filing number : CAL 3871/98

## PHYSICAL AND CHEMICAL PROPERTIES

Appearance : Colorless or clear yellow liquid  
Viscosity : 6.29 mPa.s at 20°C  
Specific gravity : 1.064-1.069 at 20°C  
Melting point : -17°C  
Boiling point : 87°C at 760 mm Hg (start of decomposition)  
Vapor pressure : 1.07 mbar at 20°C  
Flash point : 21°C (open cup)  
Solubility : Hydrolysis in water  
Soluble in DMSO  
Soluble in ethylic alcohol

## TOXICOLOGICAL INFORMATIONS AND USE SAFETY

See Material Safety Data Sheet.

## STORAGE AND DISPOSAL

Storage : In dark and at room temperature  
Expiry date : December 1999

**elf aquitaine exploration production france**



CAT/4/ECH02  
Rév. 1.0  
21.01.98

adresse postale  
B P 22 - 64170 Lacq  
téléphone : +33 (0)5 59 92 22 22  
Central telex/Lacq 560 804

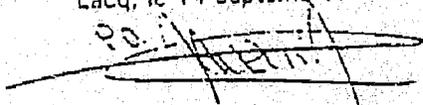
C.A.L.  
BP 108  
95, rue Danton  
92300 LEVALLOIS PERRET  
FRANCE  
A l'attention de Mr. BOURALY

Ref : AOGHJU

CERTIFICAT D'ANALYSE de l'ACIDE THIOACETIQUE (ATA)

CARACTERISTIQUES	RESULTATS
Pureté (%Pds)	99.58
Aspect	Conforme

Lacq, le 14 septembre 1998

  
Chef de service  
Centre d'Applications Techniques  
E. LOPEZ

Ref: 112  
**COMPLETE DIET**  
**RABBIT MAINTENANCE DIET**  
 Appearance: 4.5 mm diameter granules  
 Conditioning: bags of 25 kgs

Daily portion: in accordance with race and body weight, Rabbits 100-150 g, water *ad libitum*.

FORMULA %

Cereals .....	43.8
Grain byproducts and legumes .	49
Vegetable protein (soya bean meal, yeast) .....	4.2
Vitamin and mineral mixture....	3

AVERAGE ANALYSIS %

Calorific value (Kcal/kg) .....	2200
Moisture .....	10
Proteins .....	13
Lipids .....	2.7
Carbohydrates (N.F.E.) .....	49.3
Fibre .....	17
Minerals (ash) .....	8

AMINO ACID VALUES  
(calculated in mg/kg)

Arginine .....	6800
Cystine .....	2100
Lysine .....	4600
Methionine .....	1600
Tryptophan .....	1400
Glycine .....	5200

FATTY ACID VALUES  
(calculated in mg/kg)

Palmitic acid .....	6400
Palmitoleic acid .....	0
Stearic acid .....	600
Oleic acid .....	6400
Linoleic acid .....	12100
Linolenic acid .....	2400

MINERALS (calculated in mg/kg)			
	Nat. val.	CMV val.	Total
P.....	3500	3500	7000
Ca .....	4500	4500	9000
K .....	11600	0	11600
Na .....	400	1600	2000
Mg .....	2100	100	2200
Mn .....	40	40	80
Fe.....	160	140	300
Cu .....	12	15	27
Zn .....	30	45	75
Co .....	0.1	1.5	1.6
I .....	0	0	0
Cl .....	500	3000	3500

VITAMINS (calculated per kg)			
	Nat. val.	CMV val.	Total
Vitamin A	2850 IU	6500 IU	9350 IU
Vitamin D3	30 IU	1000 IU	1030 IU
Vitamin B1	4.3 mg	0 mg	4.3 mg
Vitamin B2	3.8 mg	0 mg	3.8 mg
Vitamin B3	16 mg	0 mg	16 mg
Vitamin B6	1 mg	1 mg	2 mg
Vitamin B12	0 mg	0 mg	0 mg
Vitamin E	16 mg	10 mg	26 mg
Vitamin K3	6 mg	1 mg	7 mg
Vitamin PP	55 mg	5 mg	60 mg
Folic acid	0 mg	0 mg	0 mg
Biotin	0 mg	0 mg	0 mg
Choline	850 mg	200 mg	1050 mg
Meso-Inositol	0 mg	0 mg	0 mg

Available under quality "Control Ref.: 112 C"

UAR, 7 rue Gallieni, 91360 Villemoisson - Tel : 01.69.04.03.57 - Fax : 01.69.04.81.97  
 (Ref. Doc. UAR : 1992)

2. Diet formula

**CERTIFICATE OF AUTHENTICITY**

THIS IS TO CERTIFY that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

Data produced 08 - 04 - 2000 Susan Rivera  
(Month) (Day) (Year) Camera Operator

Place Syracuse New York  
(City) (State)



**END**