TNO Triskelion

TNO Triskelion report

V 9915/11

Evaluation of eye irritation potential of Vires 5 *in vitro* using the Isolated Chicken Eye Test

Date	May 2011
Author	M.K. Prinsen
At request of	Vires 5 BvbA Biest 41 2990 Wuustwezel Belgium
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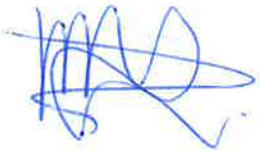
Number of appendices	3
Number of figures	1
Number of annexes	3

Statement of GLP compliance

I, the undersigned, hereby declare that this report constitutes a complete and accurate representation of the study and its results. All study activities performed by TNO Triskelion were carried out in compliance with the current OECD Principles of Good Laboratory Practice[1].
Chemical analysis for the verification of the test substance and properties was not performed in this study.

Study director

Mr. M.K. Prinsen		26-05-2011
Name	Signature	Date (dd-mm-yyyy)

[1] The OECD Principles of Good Laboratory Practice are accepted by Regulatory Authorities throughout the European Community, USA and Japan. The most recent endorsement of compliance of the Test Facility with these principles is attached to this report as Annex 2. This endorsement is valid for the indicated areas of expertise, currently part of TNO Triskelion.

Quality Assurance Statement

I, the undersigned, hereby declare that this report provides an accurate record of the procedures employed and the results obtained in this study; all audits were reported to the respective study director and management on the dates indicated.

Phase	*	Start date of audit	Date of audit report
Authorised study plan	Yes	29 April 2011	29 April 2011
Test substance application	Yes	29 April 2011	29 April 2011
Effect scoring	No	31 March 2011	31 March 2011
Histology	No	27 May 2011	27 May 2011
Draft report and study file	Yes	25 May 2011	25 May 2011
Final report	Yes	27 May 2011	27 May 2011

* This type of short-term study is carried out frequently and the Quality Assurance Unit does not audit the experimental phase of each individual study; the processes involved are audited at regular intervals according to a predetermined schedule. This column indicates whether or not the audit was of this particular study.

P.B. Davis B.A.
Quality Assurance auditor

Date : 27 MAY 2011

Contents

Summary

1. A sample of Vires 5 was evaluated neat for eye irritation potential in the Isolated Chicken Eye (ICE) test. In addition, the test included a negative control (saline) and a positive control (BAC 5%). Chicken eyes were obtained from slaughter animals used for human consumption. The isolated chicken eyes were exposed to a single application of 30 µL of the test sample for 10 seconds followed by a 20 ml saline rinse. Three main parameters were measured to disclose possible adverse eye effects: corneal thickness (expressed as corneal swelling), corneal opacity and fluorescein retention of damaged epithelial cells.

2. Vires 5 caused slight swelling of the cornea, very slight or slight corneal opacity, and slight fluorescein retention. The calculated Irritation Index was 50 (max possible score is 200).

3. The negative control (saline) caused no corneal effects.

4. The positive control BAC 5% caused severe corneal swelling, severe opacity and severe fluorescein retention. The calculated Irritation Index was 158.

5. According to the GHS and the EU-CLP classification schemes of the ICE, Vires 5 is considered to be Category 2B: “Mild irritant/causes eye irritation” (GHS classification) and Category 2: “Irritating to eyes” (borderline case to not classified; EU-CLP classification).

1 General

1.1 Study sponsor and sponsor representative

Sponsor:
Vires 5 BvbA
Biest 41
2990 Wuustwezel
Belgium

Monitor:
Mr. R. Schade
Costec
P.O. Box 51
3330AB Zwijndrecht

1.2 Testing facility

TNO Triskelion
Postal address: P.O. Box 360, 3700 AJ Zeist, the Netherlands.
Location: Utrechtseweg 48, Zeist, the Netherlands.
Phone +31 888666000; Fax +31 888668728

1.3 Responsible personnel

Study director : Mr. M.K. Prinsen[1]
Histotechnique : Ms. E.C.M. van Oostrum
Management : Ms. M.J.S.T. Steenwinkel

[1] Toxicology and Applied Pharmacology department. E-mail: menk.prinsen@tno.Triskelion.nl ; Phone +31 888661791; Fax +31 888668728

1.4 Time schedule

Experimental conduct date : 29 April 2011

2 Introduction

2.1 Objective

The purpose of this study was to provide *in vitro* data on the eye irritation potential of the test substance using the Isolated Chicken Eye (ICE) test, formerly known as the Chicken Enucleated Eye Test (CEET). The test substance was applied in one single dose onto the cornea of isolated eyes which were obtained from slaughter animals used for human consumption. In this *in vitro* bioassay, three parameters were measured to disclose possible adverse eye effects, namely corneal thickness (expressed as corneal swelling), corneal opacity and fluorescein retention. The measurement of corneal swelling in this assay provided a highly objective parameter, which enabled the investigator to discriminate the damaging effects of test substances very precisely, this in contrast to the conventional rabbit test which uses subjective macroscopic measurements only. In combination with the measurement of corneal opacity and fluorescein retention, though assessed by subjective observation, but being very accurately evaluated by use of the slit-lamp microscope, a reliable evaluation of the eye irritation potential of the test substance was achieved.
In their latest updates of the guidelines on Eye Irritancy Testing, both the EU and the OECD allow for the use of alternative *in vitro* test systems for screening or identification of severe eye irritants, in order to reduce animal use and suffering.

Furthermore, the ICE test is one of two organotypic *in vitro* assays that received statements of scientific validity from the ECVAM Scientific Advisory Committee (ESAC) to be used as screening tests for the identification of substances as ocular corrosives and severe eye irritants in a tiered testing strategy as part of a weight-of-evidence approach (ESAC 2007 at http://ecvam.jrc.it/index.htm).

2.2 Applicable guidelines

OECD guideline no. 438: "Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants", adopted on 7 September 2009.

3 Study plan and deviations

3.1 Study Plan

The study was carried out at the testing facilities of TNO Triskelion, Utrechtseweg 48, 3704 HE Zeist, the Netherlands, according to study plan P9915/11, approved by the study director on 27 April 2011.

3.2 Deviations

No deviations from the study plan occurred during this study.

4 Materials and methods

4.1 Characterization of the study substance

Date of receipt	: 26 April 2011
Test substance name	: Vires 5
Purity	: 99.69%
Lot number	: 23105
Storage conditions	: ambient
Appearance	: clear colourless liquid
Expiry date	: 1 April 2012
TNO dispense number	: 11007B

Positive control for liquids

Chemical name	: benzalkonium chloride
Appearance	: white lumps
TNO CBS number	: 37343
Manufacturer/supplier	: Sigma-Aldrich
CAS Reg. no.	: 8001-54-5
Expiry date	: 31 January 2021
Storage conditions	: ambient temperature, in the dark
Concentration tested	: 5% (w/w) aqueous

Negative control

Control substance	: physiological saline 0.9%
Supplier	: Eurovet, Bladel, The Netherlands
Batch no.	: B0807-02
Expiry date	: February 2013
Storage conditions	: ambient temperature

4.2 Characterization of the test system

Approximately 7 weeks old, male or female chickens (ROSS, spring chickens), body weight range approximately 1.5 - 2.5 kg, were used as eye-donors. Heads of these animals were obtained from poultry slaughterhouse v.d. Bor, Amersfoortseweg 118, Nijkerkerveen, the Netherlands. Heads of the animals were cut off immediately after sedation of the animals by electric shock and incision of the neck for bleeding, and before they reached the next station on the process line. The heads were placed in small plastic boxes on a bedding of paper tissues moistened with isotonic saline. Next, they were transported to the testing facility. During transportation, the heads were kept at ambient temperature.

4.3 Experimental design

Within 2 hours after kill, eyes were carefully dissected and placed in a superfusion apparatus using the following procedure: First the eye-lids were carefully removed without damaging the cornea and a small drop of Fluorescein sodium BP 2.0% w/v (Minims, Chauvin, England) was applied to the corneal surface for a few seconds and subsequently rinsed off with isotonic saline at ambient temperature. Next, the head with the fluorescein-treated cornea was examined with a slit-lamp microscope (Slit-lamp 900 BP, Haag-Streit AG, Liebefeld-Bern, Switzerland) to ensure that the cornea was not damaged. If undamaged (e.g., fluorescein retention and corneal opacity scores of ≤ 0.5), the eye was further dissected from the head without damaging the eye or cornea. Care was taken to remove the eye-ball from the orbit without cutting off the optical nerve too short.
The enucleated eye was placed in a stainless steel clamp with the cornea positioned vertically and transferred to a chamber of the superfusion apparatus (TNO, Zeist, the Netherlands). The clamp holding the eye was positioned in such a way that the entire cornea was supplied with isotonic saline from a bent, stainless steel tube, at a target rate of 0.10 - 0.15 mL/min (peristaltic pump set at speed 5.00, Watson-Marlow 205CA, Rotterdam, the Netherlands). The chambers of the superfusion apparatus as well as the saline were temperature controlled at approximately 32^{o}C (water pump set at 36.4^{o}C; Lauda 103, Germany).
After placing in the superfusion apparatus, the eyes were examined again with the slit-lamp microscope to ensure that they were not damaged. Corneal thickness was measured using the Depth Measuring Attachment No. I for the Haag-Streit slit-lamp microscope. Corneal thickness was expressed in instrument units. An accurate measurement was taken at the corneal apex of each eye.
Eyes with a corneal thickness deviating more than 10% of the average corneal thickness of the eyes, eyes showing opacity (score higher than 0.5), or were unacceptably stained with fluorescein (score higher than 0.5) indicating the cornea to be permeable, or eyes that showed any other signs of damage, were rejected as test eyes and replaced.

Three test eyes per test sample, one negative control eye and three positive control eyes were selected for testing. Each eye provided its own baseline values for corneal swelling, corneal opacity and fluorescein retention. For that purpose, after an equilibration period of 45-60 minutes, the corneal thickness of the eyes was measured again to determine the zero reference value for corneal swelling calculations.

At time t = 0 (i.e. immediately after the zero reference measurement), the following procedure was applied for each test eye: The clamp holding the test eye was placed on paper tissues outside the chamber with the cornea facing upwards.

Next, the eyes (corneas) were treated with the study substances according to the following scheme:

Group	Treatment	Exposure conditions			Number of eyes
		Volume	Duration	Rinsing	
Negative control	Saline	30 µL	10 seconds	20 mL saline	1
Positive control	5% BAC	30 µL	10 seconds	20 mL saline	3
Test group	Vires 5	30 µL	10 seconds	20 mL saline	3

After rinsing, each eye in the holder was returned to its chamber.
The eyes were examined at approximately 0, 30, 75, 120, 180 and 240 minutes after treatment, using the criteria and scoring system given in Annex 1. Fluorescein retention was only scored at approximately 30 minutes after treatment. All examinations were carried out with the slit-lamp microscope.

After the final examination, the test substance treated eyes, the negative and positive control eyes were preserved in a neutral aqueous phosphate-buffered 4% solution of formaldehyde. The corneas were embedded in paraffin wax, sectioned at 5 µM and stained with PAS (Periodic Acid-Schiff). The microscopic slides were filed in the archives and kept available for histopathological examination if considered relevant. In the case of Vires 5, histopathological examination was considered not relevant, because no borderline severe corneal effects were observed.

4.4 Evaluation of the results

In the ICE test, the eyes were examined at several time intervals after treatment to determine ocular effects using the parameters of corneal thickness (swelling), corneal opacity and fluorescein retention. Defined scoring scales were used for each parameter to define the severity of effects into four categories (I-IV). Four classes of eye irritancy (not irritating; slightly irritating; moderately irritating; severely irritating) were identified in the ICE test by combination of the categories defined for each of the evaluation parameters using the Prediction Model described in Annex 1.
Regulatory classification of eye irritants was based on the standard in vivo Draize Eye test. Various international regulatory classification systems are in use. Some systems, such as in the Globally Harmonized System (GHS), recognize four classes, viz. not classified (NC), mild irritant (Category 2B), irritant (Category 2A), and severe irritant (Category 1). The new EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP) entered into force on 20 March 2009. CLP implements the Globally Harmonised System (GHS). The EU-CLP classification system recognizes three classes, i.e. not classified (NC), irritant (Category 2) and severe irritant (Category 1).

The table below shows the criteria used in the EU-CLP regulatory classification system to identify the different classes of eye irritants.

Table - Evaluation criteria and prediction model applied within the Annex V Draize test for EU-CLP hazard classification purposes

Ocular Lesion	Mean of positive scores for ocular lesions at 24, 48 and 72h in studies employing 3 rabbits	
	Category 2	Category 1[a]
Corneal Opacity	≥1	≥3
Iris lesion	≥1	≥1.5
Conjunctival Redness	≥2	n.a.
Conjunctival Oedema	≥2	n.a.

[a] Also applies, when applied to the eye of an animal, if a substance produces at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days

In order to translate the eye irritancy scores from the ICE test to an EU-CLP regulatory classification, it is necessary to reconcile four irritancy classes from the ICE study into three classes within the EU-CLP regulatory classification scheme. This was achieved through application of the prediction model defined in Annex 1, which is based on scientific judgement and which is supported by several years of experience with conduct of the ICE test. As a result of the reconciliation of the four ICE classes of irritancy into the 3 classes of the EU-CLP regulatory classification system, it is possible that materials defined as slightly irritant using the ICE test Prediction Model may be identified in the EU-CLP regulatory classification system as not classified.

5 Results

An overall summary of the results of the test substance based on maximum mean values for corneal swelling, corneal opacity, and fluorescein retention, the irritation categories assigned to the parameters, the irritation index and the regulatory classifications tested is presented in Table 1.
The mean values for corneal swelling, corneal opacity and fluorescein retention recorded at the various observation time points for the test substance are presented in Table 2.
Individual values for corneal swelling, corneal opacity and fluorescein retention for the test substance are presented in Appendix 1.

The negative control eye did not show any corneal effect and demonstrated that the general conditions during the tests were adequate (Appendix 2).
The positive control eyes showed severe corneal effects and demonstrated the suitability and sensitivity of the ICE to detect severe eye irritants (Table 3/ Appendix 3).

6 Discussion and conclusion

Corneas of isolated chicken eyes were treated with Vires 5 at a dose volume of 30 µL for 10 seconds.
Vires 5 caused slight corneal swelling, very slight or slight corneal opacity, and slight fluorescein retention. The calculated Irritation Index was 50 (max possible score is 200).
According to the GHS and the EU-CLP classification schemes of the ICE, Vires 5 is considered to be Category 2B: “Mild irritant/causes eye irritation” (GHS

classification) and Category 2: “Irritating to eyes” (borderline case to not classified; EU-CLP classification).

7 Documentation and retention of records and specimens

The following documents and materials will be retained for 5 years:
- Raw data or true copies of these
- Correspondence
- All other information related to the study.

At the end of the retention period, the sponsor will be asked whether these documents and materials should be discarded, retained for an additional period, or transferred to the archives of the sponsor.
The master copy of the final report, the approved study plan and any amendments thereof will be retained for at least 15 years.
Microscopic slides will be retained for at least 15 years and then removed from the archives. Unless otherwise agreed, remaining test substance will be retained for at least one month and then discarded.
Documents and materials will be retained in the archives of TNO located in Zeist. The archiving period starts on the cover date of the final report.

8 References

- Koëter, H.B.W.M. and M.K. Prinsen. Comparison of in vivo and in vitro eye irritation test systems: A study with 34 substances. Alternative Methods in Toxicology, Vol. 3, A.M. Goldberg, 1985, Mary Ann Liebert, Inc., New York.
- Koëter, H.B.W.M. and M.K. Prinsen. Validation of an in vitro eye irritation study; A first step. Alternative Methods in Toxicology, Vol. 5, A.M. Goldberg, 1987, Mary Ann Liebert, Inc., New York.
- Prinsen, M.K. and H.B.W.M. Koëter. Justification of the Enucleated Eye Test with eyes of slaughterhouse animals as an alternative to the Draize Eye Irritation Test with rabbits. Food and Chemical Toxicology, Vol. 31, no. 1, pp. 69-70 (1993).
- Prinsen, M.K. The Chicken Enucleated Eye Test (CEET): a practical (pre)screen for the assessment of eye irritation/corrosion potential of test materials. Food and Chemical Toxicology, Vol. 34, no. 4, 1996.

Table 1 - Summary results of the slit-lamp examination

Test material	Maximum mean score for:			Irritation categories[1]	Irritation Index[2]	Classifications (EU-CLP[3]/GHS[4])
	Swelling %	Opacity	Fluorescein retention			
Vires 5	16	0.7	1.0	II;II;II	50	2[6]/2B
Saline (negative control)	0	0.0	0.0	Not applicable; one eye tested		
BAC 5% (w/v) (positive control)	38	3.0[5]	3.0	IV;IV;IV	158	Category 1

1 I = no effect; II = slight effect; III = moderate effect; IV = severe effect.

2 Irritation Index = maximum mean corneal swelling + maximum mean opacity (x 20) + mean fluorescein score (x 20)

3 EU-CLP: NC = not classified; Category 2 = Irritating to eyes; Category 1 = irreversible effects on the eye/serious damage to the eye. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

4 GHS: NC = not classified; Category 2B = mild irritant, causes eye irritation; Category 2A = irritant, causes eye irritation; Category 1 = irreversible effects on the eye/serious damage to the eye. United Nations-Economic Commission for Europe (UN/ECE) (2003). Globally Harmonised System of Classification and Labelling of Chemicals (GHS). UN, New York and Geneva, 2007.

5 Severe loosening of epithelium (blistering)

6 Borderline with not classified

Table 2 - Mean values and irritancy categories: Vires 5

Time intervals [min]	Swelling %	Opacity	Fluorescein
30	2	0.5	1.0[1]
75	7	0.5	
120	13	0.5	
180	14	0.5	
240	16	0.7	

Parameter	Maximum score	Irritancy category
Swelling %	16	II
Opacity	0.7	II
Fluorescein retention	1.0	II

1 fluorescein measurement at t = 30 min only

The criteria and scoring system is given in Annex 1.

Table 3 - Mean values and irritancy categories: BAC 5%

Time intervals [min]	Swelling %	Opacity	Fluorescein
30	9	3.0	3.0[1]
75	16	3.0	
120	27	3.0	
180	31	3.0	
240	38	3.0	

Parameter	Maximum score	Irritancy category
Swelling %	38	IV
Opacity	3.0	IV
Fluorescein retention	3.0	IV

1 fluorescein measurement at t = 30 min only

The criteria and scoring system is given in Annex 1.

Appendix 1 - Individual values Vires 5

Eye number	Swelling %	Opacity	Fluorescein
after 30 min			
1	2	0.5	1.0
3	0	0.5	1.0
5	3	0.5	1.0
after 75 min			
1	5	0.5	
3	7	0.5	
5	8	0.5	
after 120 min			
1	12	0.5	
3	12	0.5	
5	15	0.5	
after 180 min			
1	12	0.5	
3	14	0.5	
5	15	0.5	
after 240 min			
1	13	0.5	
3	14	0.5	
5	20	1.0	

Appendix 2 - Individual values negative control saline

Eye number	Swelling %	Opacity	Fluorescein
after 30 min			
7	0	0.0	0.0
after 75 min			
7	0	0.0	
after 120 min			
7	0	0.0	
after 180 min			
7	0	0.0	
after 240 min			
7	0	0.0	

Appendix 3 - Individual values positive control BAC 5%

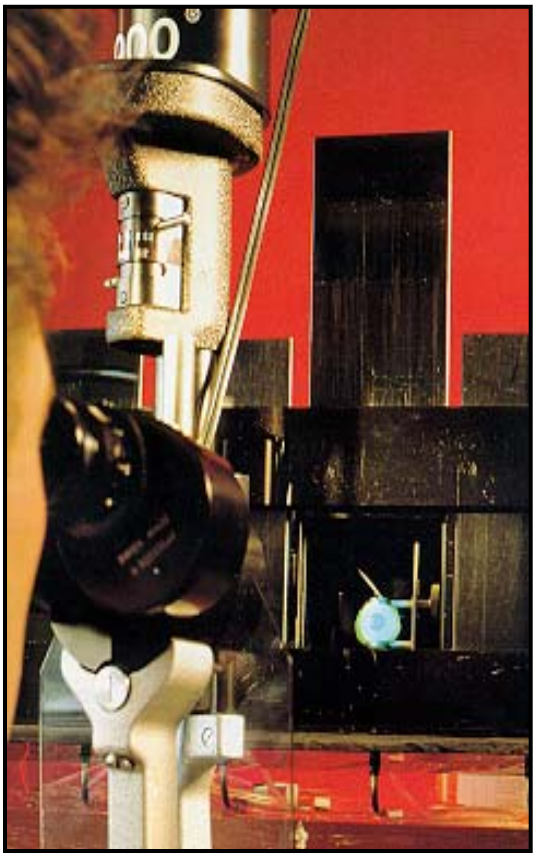
Eye number	Swelling %	Opacity	Fluorescein
	after 30 min		
2	13[1]	3.0	3.0
4	10[1]	3.0	3.0
6	5[1]	3.0	3.0
	after 75 min		
2	16	3.0	
4	13	3.0	
6	18	3.0	
	after 120 min		
2	31	3.0	
4	23	3.0	
6	28	3.0	
	after 180 min		
2	34	3.0	
4	28	3.0	
6	31	3.0	
	after 240 min		
2	39	3.0	
4	34	3.0	
6	41	3.0	

[1] severe loosening of epithelium (blistering)

Figure 1 - Experimental set-up

Superfusion apparatus

Observation of corneal effects with the slit-lamp microscope



Annex 1 - Criteria and scoring system

The following criteria and scoring systems are applied for the assessment of possible effects:

Corneal swelling

Corneal swelling, expressed as a percentage, is calculated according to the following formula:

"Corneal thickness at time t minus corneal thickness at time t = 0, divided by corneal thickness at time t = 0 and multiplied by 100".

A negative swelling up to -5% (not unusual for control eyes) will be presented as 0 % swelling in the tables/appendices.

The mean percentage of swelling for the three test eyes will be calculated for each of the observation time points of 30, 75, 120, 180, and 240 minutes. The maximum mean percentage (can be at any of the time points) will be used for classification into one of four categories (see next table page).

Corneal opacity

Opacity degree of density (area most dense taken for scoring)
0 = no opacity
0.5 = very faint opacity (= very slight)
1 = scattered or diffuse areas, details of iris clearly visible (= slight)
2 = easily discernible translucent area, details of iris slightly obscured (= moderate)
3 = severe corneal opacity, no specific details of iris visible, size of pupil barely discernible (= severe)
4 = complete corneal opacity, iris invisible (= very severe)
The mean corneal opacity value for all test eyes is calculated for the observation time points of 30, 75, 120, 180, and 240 minutes.
NOTE. In case of score 4, the thickness assessment will not be possible. Intermediate scores can also be assigned.

Fluorescein retention

0 = no fluorescein retention
0.5 = very minor single cell staining (= very slight)
1 = single cell staining scattered throughout the treated area of the cornea (= slight)
2 = focal or confluent dense single cell staining (= moderate)
3 = confluent large areas of the cornea retaining fluorescein (= severe)
Intermediate scores can also be assigned. The mean fluorescein retention value for all test eyes is calculated for the observation time point of 30 minutes only. If desired or in case of test substances that have adhered to the cornea, fluorescein retention can be determined at t=240 min or whenever the test compound is removed.

Morphological effects

These include "pitting" of corneal epithelial cells, "loosening" of epithelium, "roughening" of the corneal surface and "sticking" of the test substance to the cornea. These findings can vary in severity and may occur simultaneously. The classification of these findings is subject to the interpretation of the investigator.

Microscopic effects

Corneal lesions are determined by microscopical examination. The effects include but are not limited to erosion, necrosis and vacuolation of the epithelium, disorder of stromal fibers, pyknotic nuclei in the stroma and necrosis of the endothelium. The classification of these findings is subject to the interpretation of the investigator.

On the basis of the severity of the observed findings for corneal swelling, corneal opacity and fluorescein retention, the effects are divided into four categories, viz. I = no effect; II = slight effect; III = moderate effect; IV = severe effect.

Interpretation of corneal swelling, corneal opacity, and fluorescein retention and categorisation into the four categories is done according the following methodology:

Corneal swelling:

Mean corneal swelling (%)		Category
-5 - 5		I
6 - 12		II
13 - 18	(>75 min. after treatment)	II
	(≤75 min. after treatment)	III
19 - 26		III
27 - 32	(>75 min. after treatment)	III
	(≤75 min. after treatment)	IV
>32		IV

Corneal opacity:

mean max. opacity score	Category
0.0 - 0.5	I
0.6 - 1.5	II
1.6 - 2.5	III
2.6 - 4.0	IV

Fluorescein retention:

mean fluorescein retention score at 30 min after treatment:	Category
0.0 - 0.5	I
0.6 - 1.5	II
1.6 - 2.5	III
2.6 - 3.0	IV

On the basis of the mean scores an Irritation Index can be calculated to allow for numerical ranking and comparison. The index is based on the addition of the maximum mean scores obtained for the parameters according to the following formula:
Irritation Index = Maximum Mean Corneal Swelling + Maximum Mean Opacity (times 20) + Mean Fluorescein Score (times 20).
A factor of 20 is included to give equal weight to the scores obtained for opacity and fluorescein retention in the index compared to the maximum swelling possible (circa 60%). The maximum Irritation Index possible is circa 200.

ASSESSMENT OF THE GENERAL *IN VITRO* EYE IRRITANCY AND REGULATORY GHS CLASSIFICATION

The *in vitro* irritancy is assessed by reading the irritancy class that corresponds to the combination of the category obtained for corneal swelling, corneal opacity and fluorescein retention, which are presented in the scheme below.

Irritancy Class (Regulatory GHS Classification)	Combinations of the three categories
A. NC = not classified	3 x I 2 x I, 1 x II 2 x II, 1 x I
B. Slightly irritating (GHS[3] category 2B: *Mild irritant/ causes eye irritation*)	3 x II 2 x II, 1 x III 1 x I, 1 x II, 1 x III[1]
C. Moderately irritating (GHS category 2A: *Irritant/ causes eye irritation*)	3 x III 2 x III, 1 x II 2 x III, 1 x I 2 x I, 1 x IV[1] 2 x II, 1 x IV[1] 2 x III, 1 x IV[2] 1 x II, 1 x III, 1 x IV[1]
D. Severely irritating (GHS category 1: *Irreversible effects on the eye/serious damage to the eye*)	3 x IV 2 x IV, 1 x III 2 x IV, 1 x II[1] 2 x IV, 1 x I[1] immediate corneal opacity score 3 in at least 2 corneas corneal opacity score 4 in at least 2 corneas severe loosening of epithelium in at least one cornea

[1] Combinations of categories less likely to occur.

[2] The combination of 2 x III, and 1 x IV can be considered as a borderline case between moderately irritating and severely irritating.

[3] United Nations-Economic Commission for Europe (UN/ECE) (2003). Globally Harmonised System of Classification and Labelling of Chemicals (GHS). UN, New York and Geneva, 2007 (http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html).

EU-CLP CLASSIFICATION (CLP) OF EYE IRRITANTS; EXTRAPOLATION FROM *IN VITRO* RESULTS TO *IN VIVO* CLASSIFICATION

Extrapolation from the *in vitro* irritancy results to the EU-CLP classification will be carried out using scientific judgement, re-dividing the original four irritancy classes to three. The combinations of the three categories that are allowed for each of the three classifications are mentioned in the scheme below.

Regulatory Classification	Combinations of the three categories
NC = not classified	3 x I 2 x I, 1 x II 2 x II, 1 x I
EC/GHS Category 2[4]: *Irritating to eyes*	3 x II[3] 2 x II, 1 x III 1 x I, 1 x II, 1 x III[1],[3] 3 x III 2 x III, 1 x II 2 x III, 1 x I 2 x I, 1 x IV[1] 2 x II, 1 x IV[1] 2 x III, 1 x IV[2] 1 x II, 1 x III, 1 x IV[1]
EU-CLP Category 1[4]: *Irreversible effects on the eye/ serious damage to eyes*	3 x IV 2 x IV, 1 x III 2 x IV, 1 x I[1] 2 x IV, 1 x I[1] immediate corneal opacity score 3 in at least 2 corneas corneal opacity score 4 in at least 2 corneas severe loosening of epithelium in at least one cornea

[1] Combinations of categories less likely to occur.

[2] The combination of 2 x III, and 1 x IV can be considered as a borderline case between irritating and severely irritating.

[3] These combinations can be considered as a borderline case between not irritating and Category 2.

[4] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Annex 2 - Endorsement of GLP compliance

voedsel en waren autoriteit

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF
GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 2004/9/EC the conformity with the OECD Principles of GLP was assessed on 27-31 October 2008 at

TNO Quality of Life
Utrechtseweg 48, 3704 HE Zeist
P.O. Box 360, 3700 AJ Zeist

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following areas of expertise: Toxicity, mutagenicity, analytical and clinical chemistry, kinetics and metabolism, safety pharmacology, worker exposure and in-vitro studies.

Den Haag, 03 February 2009

Dr Th. Helder
Manager GLP Compliance Monitoring Program

Food and Consumer Product Safety Authority (VWA)
Prinses Beatrixlaan 2, 2595 AL Den Haag
Postbus 19506, 2500 CM Den Haag, The Netherlands

Annex 3 – Test substance information

Analysecertificaat voor product: Vires5 Deso

Chargenr :
MHD :

Art. Nr.

Samenstelling
H_2O 99,69%, NaCL 0,26%, HCLO + OCL 0,05%

Eigenschappen

Omschrijving	*Waarde*
Geur	karakteristiek
Kleur	transparant
Vorm	vloeibaar
pH Waarde	pH 6,95 – 7,05
Redox Waarde	OrpV 750 - 790
Vriespunt	< 0 °C
Kookpunt	100 °C
Vlampunt °C	n.v.t.
Explosieve eigenschap	n.v.t.
Soortelijk gewicht	1,002 kg/liter (4^oC)
Stabiliteit	stabiel onder normale omstandigheden

Overige informatie

De informatie op dit Analysecertificaat is naar ons beste weten correct en in overeenstemming met de algemene wetenschappelijke – en technische kennis ten tijde van uitgifte. Vires5 BvbA kan echter geen aansprakelijkheid accepteren over verlies, schade of letsel resulterend uit het gebruik. Bij het samenstelling van dit blad hebben wij alle geëigende toepassingen van het product die ons bekend zijn in aanmerking genomen. Iedere gebruiker van dit product dient ons bij elke nieuwe of ongebruikelijke toepassing te raadplegen. Wij maken u erop attent dat iedere tussenpersoon/leverancier verantwoordelijk is dat dit Analysecertificaat aan de eindgebruiker wordt doorgegeven. Indien de eindgebruiker het gewenst acht dat dit Analysecertificaat rechtstreeks aan hem of haar wordt toegezonden, zullen wij, indien wij hieromtrent worden geïnformeerd, dit verzorgen.