

# The HET-CAM, a Reliable *In Vitro* Test for the Prediction of Eye Irritation

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## Introduction

One of the most important biological properties of consumer products like cleansing agents or cosmetics is the local compatibility to skin and to mucous membranes. For the evaluation of these toxicological properties, standardized *in vivo* tests are accepted by public health authorities as being valid to estimate the irritation potential of chemicals and being suitable for any risk assessment. During the last decade HENKEL has established a useful strategy to evaluate the local compatibility, especially to mucous membranes without any animal test. One of the most important part of this test strategy is the so-called HET-CAM, the *in vitro* assay on the chorioallantoic membrane of fertilized chicken EGGS.

Impressed by the interest to increase the sensitivity of irritation tests, to discriminate weak as well as severe irritants, the HET-CAM has been established and integrated in our routine experimental work. The performance of this particular *in vitro* test is supported by the following important aspects:

- 1) Reproducibility
- 2) Reliability in prediction of severe as well as weak irritation properties
- 3) Reduction of animal tests
- 4) Reduction of costs and time.

## The HET-CAM

Hen's egg test on the chorioallantoic membrane

### fertilized chicken eggs

incubation

removing the top of the egg shell

### preparation of the CAM

application of the test substance

time recording of standard irritation effects:

- haemorrhage
- lysis of the vessels
- protein coagulation

## Description of the Test Method

**Biological target**  
The eggs (Hen's Laying, Stage: Stress 385 or Bantam) are incubated in an incubator fitted with automatically rotating axes in constant conditions (temperature: 37.5 °C, humidity: 40 - 60 % for nine days). The CAM is prepared by carefully removing the white inner egg membrane on day 10.

**Exposure**  
300 µl of test substances were applied to the CAM and the irritation effects were observed continuously over a five minute time period (Reaction-Time Method or discontinuous (E)C<sub>50</sub> Assay).

**End-Points**  
The parameters haemorrhage, lysis of the blood vessels and coagulation (proteinaceous and/or extravasation) or an increase in the CAM opacity were used in order to evaluate the reaction-time method; the quotient (Q) of the irritation index of the test substance and that of the reference substance is calculated. In case of acute and/or topical test substances, the compound is mixed with evaluated semi-quantitatively (Q) calculated by the scores of the external standard.

**Control**  
Every day the test was carried out on the laboratory (reference) species: rabbits, the mouse, surfactant "phosphor" AS<sup>®</sup> (human reagentum *Na<sub>2</sub>Phosphor-Adiponitrat*), and some reference eyes.

**Restrictions**  
The only restriction of the HET-CAM protocol are sticky and viscous compounds and those reference eyes.

## Scoring Systems of the HET-CAM

Reaction-Time Method

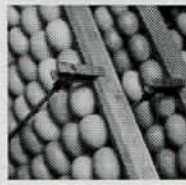
ICI score	slightly irritating	moderately irritating	severely irritating
>0.8, <1.2	X		
≥1.2, <2.0		X	
≥2.0			X

There is a cut-off point for statistics analysis at Q = 2.5

End-Point Assessment

ISI score	slightly irritating	moderately irritating	severely irritating
<4			
5, ≤ 12	X		
13, < 16		X	
≥16			X

ISI score of the most impressive irritation parameter. The reason is only visible after an exposure period of 30 sec.



## Prediction of Irritating Properties

**In Vitro Class**  
severely irritating  
irritating

moderately irritating  
slightly irritating

1  
63

1  
1

4

15  
7  
1  
1

slightly irritating  
moderately irritating  
irritating  
severely irritating

**In Vivo Class**

## Conclusion

The HET-CAM seems to be much more than only as a screening test for raw materials, but also for the evaluation of the irritation potential of formulated products.

The presented data of the HET-CAM demonstrate the high correlation between *in vivo* and *in vitro* data for mild as well as for severe irritants. All shown *in vivo* test results have been requested by official authorities during notification according to the Chemical Law.

Based on our experience over the last 10 years, we think that the HET-CAM is able to give a good prediction of the eye irritation potential of test substances.

To compare *in vitro* and *in vivo* results, it is important to have in mind, that the eye irritation is a complex process with different effects on different tissues. The HET-CAM is much less complex, but this alternative method was developed to predict the total eye irritation potential in the sense of a class: it could be difficult to focus the interest of the prediction of specific tissue effects in rabbit's eye. Nevertheless, this *in vitro* assay has been demonstrated to be sufficient for the prediction of the eye irritation potential over a broad spectrum of chemistry and irritating properties. The test is recommended as a useful and important tool for the risk assessment as well as for the safety evaluation.

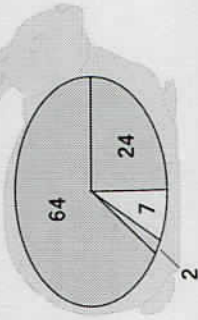
## Classification Scheme of *In Vivo* Scores

Irritation properties	Mucous membranes		Iris		Cornea opacity	
	eyelid	conjunctiva	eyelid	conjunctiva	eyelid	conjunctiva
slight	<2.0	61.0	43	0	0	0
moderate	2.0	41.0	43	<1.0	<1.0	64
irritating	>2.0	<41.0	>43	>1.0	>1.0	>64
severe	>2.5	<42.0	>43	>1.0	>1.0	>64
severe	>2.5	<42.0	>43	>1.5	>1.0	>64

The listed scores represent the scores averaged after 24, 48 and 72 hours and the total number of irritations, or recovered for average scores of at least 20% of those test three results were chosen.

## *In Vivo* Classification of Tested Articles

- slightly irritating
- moderately irritating
- irritating
- severely irritating



## Tested Articles

number of test compounds:	97
physical appearance:	70 liquids, 6 creams and 21 solids
solubility in water:	35 soluble, 5 non-soluble, 55 not specified
chemical classes:	alkali sulfate
inorganic compounds:	fatty alcohols, ketones, acids, esters and ethers, fatty acid amides and amines, triglycerides, sulfo-succinates, betains and sulfonates
aliphatic compounds:	quinones, benzoic acid esters, phthalic acid esters, phosphonic acid esters, polycyclic ethers
alicyclic compounds:	triazines, vitamins
heterocyclic compounds:	glucosides
cation hydrogen derivatives:	protein hydrolyzates, polyacrylates
polymers:	fragrances, hardydes
cosmetic raw materials:	