



scantox

TEST REPORT

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SPONSOR:

InterHealth AB
Kungsängsvägen 27
S-561 51 Huskvarna
Sweden

ECOMER ALKYLGLYCEROLS

HEMOLYSIS TEST (Direct Contact Test)

AUTHOR:

C Nicholas Edwards, BSc, PhD

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SCANTOX DK-4623 Lille Skensved Denmark Tel. +45 56 86 15 00 Fax +45 56 82 12 02

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The investigation described in this report "Ecomer Alkylglycerols - Hemolysis Test (Direct contact test)" was carried out under my supervision and responsibility and in accordance with the OECD Principles of Good Laboratory Practice (as revised in 1997), which are essentially in conformity with:

EEC Principles of Good Laboratory Practice, Directive 87/18/EEC,
United States Food and Drug Administration, Title 21, CFR, Part 58, and
Japanese Ministry of Health and Welfare, PAB Notification No. 424.

The report is a complete and accurate account of the methods employed and the data obtained.

SCANTOX
27 May 1999



C Nicholas Edwards, BSc, PhD
Study Director

QUALITY ASSURANCE STATEMENT

The Quality system at Scantox complies with the OECD principles of Good Laboratory Practice and the European Standards EN45001.

Short term routine studies of the type described in this report "Ecomer Alkylglycerols - Hemolysis Test (Direct contact test)" are inspected by the Quality Assurance Unit in compliance with the principles of Good Laboratory Practice. Process-based inspections are carried out regularly. Inspection reports have been communicated to the Study Director and to the management of Scantox.

Date of most recent inspection: 20 November 1998

Date of report to Study Director and management: 20 November 1998

This report has been audited by the Quality Assurance Unit and was found to be an accurate description of the methods and procedures used during the conduct of the study and an accurate reflection of the raw data.

Date of final audit: 27 May 1999


27 May 1999



Susanne B Nissen, MSc
QA Auditor

PERSONNEL RESPONSIBLE FOR THE STUDY

Study Director


C Nicholas Edwards, BSc, PhD

Quality Assurance


Susanne Benn Nissen, MSc

Sponsor Monitor


Stellen Ölmeskog

SUMMARY

“Ecomer Alkylglycerols” was tested for *in vitro* hemolysis activity (lysis of erythrocytes) according to the ISO 10993-1 guideline (1st edition, 1992) which is identical to EN 30993-1. The test was designed according to the recommendation of the Material Science Institute (MSI), Tennessee, USA (1979). The test is a “direct contact test” where the blood cells and test article are present together in an isotonic saline solution.

Aliquots of the test article oil (1 g each) were incubated with 5 ml isotonic saline (0.9% NaCl) at 37°C, corresponding to an ratio of 0.2 g/ml. After 30 minutes, blood was added to the incubation mixture (100 µl) and gently but thoroughly mixed. The incubation was maintained for a further 60 minutes. Negative controls (isotonic saline) and positive controls (distilled water) were included, and all treatments were performed in triplicate.

At the end of the incubation period, the mixtures were centrifuged for 5 minutes at 500 x g and the absorbance of the supernatant liquids was measured at 545 nm (OD_{545}).

The amount of hemolysis was calculated as $(OD_{test} - OD_{neg.cont})$ as a percentage of $(OD_{pos.cont} - OD_{neg.cont})$. Up to 5% hemolysis is considered a negative result.

The test article, Ecomer Alkylglycerols, was found to induce -0.03% hemolysis under the experimental conditions employed in this study. Thus, the test article passed the MSI hemolysis test requirements (hemolysis < 5.0%).

INTRODUCTION

The objective of the Hemolysis test is to determine whether the test article can cause lysis of red blood cells (erythrocytes). The test for haemocompatibility was performed according to the ISO 10993-1 guideline (1st edition, 1992) which is identical to EN 30993-1. The test was designed in accordance with the recommendations from the Material Science Institute (MSI), Tennessee, USA (1979).

The experimental work was performed on 26 May 1999.

This report describes the procedures used and the results obtained.

MATERIALS AND METHODS

Test article

Ecomer Alkylglycerols

Synonym: Ecomer shark liver oil

Chemical name: Diesters of alkylglycerols

Batch No: 990106

Expiry: 01/2002

Intended use: Immune system stimulator

Description: A light yellow oil

The test article was received from the Sponsor on 12 May 1999. Two blister cards were supplied, each with 30 transparent soft gelatine capsules, each containing 250 mg of the test article.

Test article characterization (purity, solubility and stability etc.) was the responsibility of the Sponsor. The test article was labelled with the laboratory number of this study and kept at room temperature in the dark. The test results relate to the above mentioned test article supplied by the Sponsor.

Isotonic saline (0.9% NaCl, Batch No 99A07S04) used for the extraction and distilled water (Batch No 98F15S10) for the positive control were purchased from Pharmacia & Upjohn A/S, Denmark.

Rabbit blood

Shortly before initiation of the test a sample of blood was collected from the ear vein of a healthy adult rabbit. A glass tube was used for blood collection and heparin (Batch No C7223B, purchased from Leo, Denmark), was added to about 50 IE/ml. The rabbit used was an SPF albino rabbit, weight 2.6 kg, 9 months old) of the stock Mol:Russian from M & B, Ejby, DK-4623 Lille Skensved, Denmark. The animal was earmarked on arrival with National Wing Bands.

Housing

The rabbit used for the study was housed in an animal room provided with filtered air at a temperature of $21^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and relative humidity of $55\% \pm 15\%$. The room was designed to give 10 air changes per hour. The room was illuminated to give a cycle of 12 hours light and 12 hours darkness. The light was on from 0600 h to 1800 h.

The animal was kept in a PPO/HIPS (Noryl®) cage (floor area; 2576 cm²) with a perforated floor. The tray under the floor was cleaned 2 - 3 times a week.

Diet

A pelleted complete diet "Altromin 2123" from Chr. Petersen, DK-4100 Ringsted was available *ad libitum*. Analyses for major nutritive components and relevant possible contaminants are performed regularly on the diet. Certificates of analyses are retained.

Drinking water

The animal had free access to bottles with domestic quality drinking water acidified with hydrochloric acid to pH 2.5 in order to prevent microbial growth. Analyses for relevant possible contaminants are performed regularly. Certificates of analyses are retained.

Blood cell suspension for test

Shortly before the test, the blood was diluted with isotonic saline so that complete hydrolysis under the test conditions would produce absorbance values of approximately 0.9 - 1.0.

Hemolysis test procedure

Aliquots of the test article oil (1 g each) were incubated with 5 ml isotonic saline (0.9% NaCl) at 37°C, corresponding to a ratio of approximately 0.2 g/ml. After 30 minutes, blood was added to the incubation mixture (100 µl) and gently but thoroughly mixed. The incubation was maintained for a further 60 minutes. Negative controls (isotonic saline) and positive controls (distilled water) were included. All control and test article treatments were performed in triplicate.

At the end of the incubation period, the incubation mixtures were centrifuged for 5 minutes at 500 x g. The absorbance of the supernatant liquids was measured at 545 nm (OD₅₄₅) using a spectrophotometer.

The percentage of hemolysis was calculated as:

$$\frac{(OD_{\text{test}} - OD_{\text{neg.cont}})}{(OD_{\text{pos.cont}} - OD_{\text{neg.cont}})} \times 100\%$$

Up to 5% hemolysis was considered a negative result.

Archives

For a period of 10 years the following material relating to the study will be retained in the archives of Scantox:

Protocol and correspondence
Test material receipts
All original data
Final report

At the end of the storage period Scantox will contact the Sponsor for instructions whether the material should be transferred, retained or destroyed.

RESULTS AND CONCLUSION

The test for haemocompatibility was performed according to the ISO 10993-1 guideline (1st edition, 1992) which is identical to EN 30993-1. The test was designed in accordance with the recommendation of the Material Science Institute, Tennessee, USA.

The test article, Ecomer Alkylglycerols, was found to induce -0.03% hemolysis (see Table 1) under the experimental condition employed in this study. Thus, the test article passed the MSI hemolysis test requirements (hemolysis <5.0%).

ECOMER ALKYLGLYCEROLS

HEMOLYSIS TEST

	Absorbance 545 nm			Mean value	- negative control	Hemolysis (%)
	I	II	III			
Negative control	0.0120	0.0130	0.0140	0.0130		
Positive control	1.0090	1.0180	1.0180	1.0150	1.0020	
Ecomer Alkylglycerols	0.0070	0.0130	0.0180	0.0127	-0.0003	-0.03%