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Bayreuth, May 16th 2010

Statement of the validity of cytotoxicity tests of the company CYTOX according to ISO 10993-5 (1999) compared to the requirements of the actual ISO 10993-5 (2009)

The cytotoxicity tests performed by CYTOX according to ISO 10993-5 (1999) in the years 2009 and older are also valid according to the actual normative revision ISO 10993-5 (2009):
The tests according to ISO 10993-5 (1999) were performed, whenever technical possible, in a quantitative manner as suggested by the actual revision ISO 10993-5 (2009).
The comparability of CYTOX cytotoxicity test results with other CYTOX cytotoxicity tests and with test results of other laboratories is possible because of the percentaged description of the results compared to the negative controls of the test procedure.
All tests were performed using adequate negative and positive controls
The extraction methods and extraction conditions used are in conformity with the actual normative revision.
The quantitative evaluation of cytotoxicity measurements are also in conformity with the actual normative revision:
According to the actual ISO 10993-5 (2009) reduction of the cell viability by no more than 30 % compared to a negative control results to the statement that the product tested is evaluated as "not cytotoxic".
The requirement of CYTOX test reports according to ISO 10993-5 (1999) were even 10 % higher by no more than 20 % cell viability reduction compared to the negative control.
Therefore the following CYTOX cytotoxicity tests according to ISO 10993-5 (1999) are also valid according to ISO 10993-5 (2009):

Test report number:
ISO 200402-00251A
ISO 200402-00251B
ISO 200501-00332
ISO 200402-00251A_V2
ISO 200510-00400

These cytotoxicity tests were performed on behalf of the company:
Sioen Fabrics s.a.
Avenue Urbino 6, Z.I. du Blanc Ballot

B-7700 Mouscron, Belgien

Dr. D. Scheddin (CEO CYTOX)

Prüfbericht

Test auf Toxizität im Materialextrakt

Prüfberichtsnummer: ISO 200501-00332

Durchgeführt im Auftrag von:

Sioen Fabrics s.a.
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Prüfobjekt:

24.02.05

Gewebe mit Bravo FR PU-Beschichtung

Eingang des Prüfmaterials: 31.01.05

Durchführung der Prüfung: 13.02.05

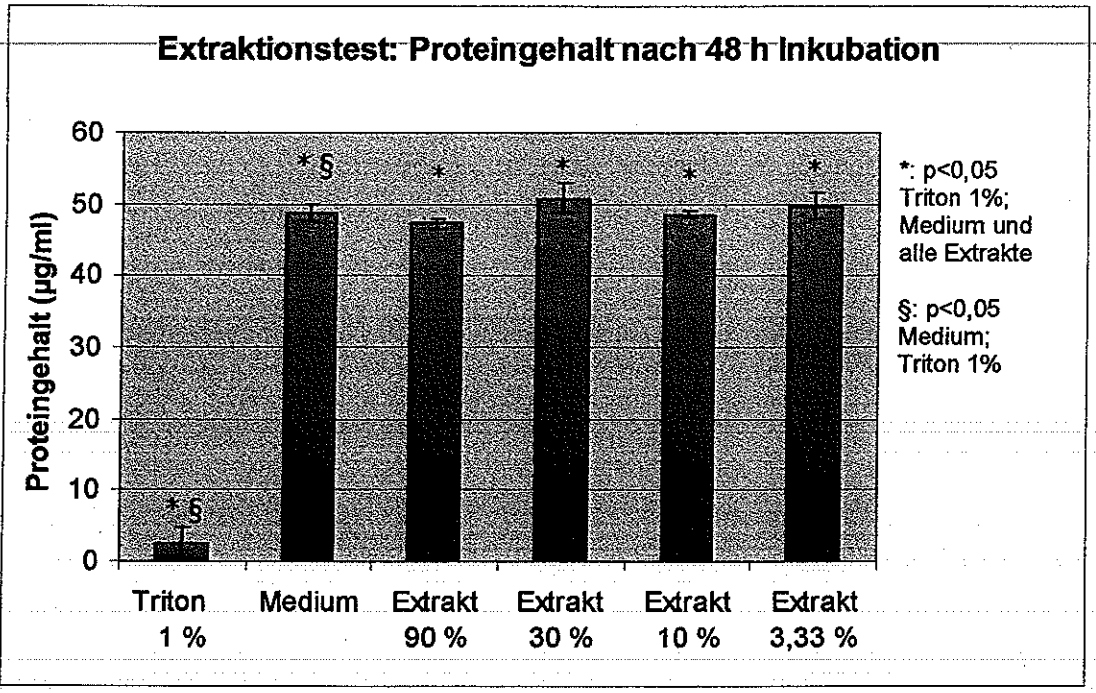
Ergebnis: **Das Gewebe mit Bravo FR PU-Beschichtung wirkt nicht zytotoxisch.**

Beschreibung und Durchführung der Prüfung:

Angewandte Norm(en): ISO 10993-5 (1999); EN 30993-5: (1994)

Das Material wurde insteril 48 h bei 37°C und 5 % pCO_2 in Extraktionsmedium (DMEM inklusive Antibiotika ohne FCS) extrahiert. Das Oberflächen-Volumen-Verhältnis betrug 2 cm^2/ml Extraktionsmedium. Nach Abschluss der Extraktion wurde das Extraktionsmedium sterilfiltriert und mit sterilem FCS supplementiert (Endkonzentration: 10 % FCS im Extraktionsmedium). Das FCS-supplementierte Extraktionsmedium wurde steril auf vorkultivierte L929-Mausfibroblastenzellen gegeben und 48 h bei 37°C, 5 % pCO_2 inkubiert. Der Extrakt wurde in vier Verdünnungsstufen (90 %, 30 %, 10 % und 3,3 %) vierfach parallel geprüft.
Als toxische Positivkontrolle wurde Triton X 100 verwendet, welches zu L929-Zellen zugegeben wurde (Endkonzentration 1 %). Als nichttoxische Negativkontrolle diente Zellkulturmedium. Gemessen wurde der Proteingehalt nach der Bradfordmethode.

Ergebnisse:



Messwerte µg/ml	Proteingehalt n=4					
	Triton 1 %	Medium	Extrakt 90 %	Extrakt 30 %	Extrakt 10 %	Extrakt 3,3 %
Mittelwert	2,40	48,75	47,24	50,83	48,62	49,73
Standardabw.	2,30	1,14	0,83	2,12	0,39	1,81

Der Wert der Positivkontrolle (Triton X 100, 1 %) liegt mit 4,9 % des Negativkontrollwertes (Medium) im gültigen Bereich unterhalb von 35 % relativ zur Kulturmediumkontrolle.
Materialien, deren Extrakte eine Verminderung des Proteingehalts von L929-Zellen um mehr als 19 % relativ zur Negativkontrolle bewirken, werden als zytotoxisch bewertet. Dies ist bei keiner Extraktkonzentration der Fall. Der Extrakt zeigt keine zytotoxische Reaktion.

Ergebnis: **Das Gewebe mit Bravo FR PU-Beschichtung wirkt nicht zytotoxisch.**

Erläuterungen / Interpretationen:

keine

Prüfung durchgeführt von: Dietmar Scheddin

genehmigt durch: Dietmar Scheddin
(Dr. D. Scheddin / Geschäftsführer CYTOX)

WICKHAM LABORATORIES

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BIOLOGICAL EVALUATION OF

BRAVO

FOR SKIN IRRITATION

BS EN ISO 10993-10:1996

FINAL REPORT

Study Director

Scott J Ridgway

To:

Sioen Fabrics
Avenue Urbino 6
Z.I. Du Blanc Ballot
B-7700 Mouscron
Belgium

From:
Wickham Laboratories Limited
Winchester Road
Wickham
Fareham
Hampshire
PO17 5EU

Master Schedule No: 1011
Internal File No: 00190405.R

Appendices (pages): None

CONTENTS

COMPLIANCE STATEMENT

As Study Director, I take responsibility for the validity of results generated in the course of these studies and confirm that the work was conducted in compliance with the principles of Good Laboratory Practice as set forth in "The Good Laboratory Practice Regulations 1999".

S J Ridgway, HNC

Section Head, Toxicology

S. J. Ridgway

Date: 16th July 2001.

MANAGEMENT STATEMENT

I have reviewed this report and concur with its contents.

C A Bishop, B.Sc., C.Chem., M.R.S.C.

Technical Director

C A Bishop

Date: 18 July 01

QUALITY ASSURANCE STATEMENT

The reported results reflect the raw data produced during the study. Inspections and reviews were made by the Quality Assurance Unit and reported to Management and the Study Director as follows:-

Inspection of study plan	31 May 2001
Inspection of technical procedures	
Preparation of extract in SCI	08 Jun 2001
Administration of extracts	12 Jun 2001
Observation and scoring at 24 hours	13 Jun 2001
Observation and scoring at 48 hours	14 Jun 2001
Observation and scoring at 24 hours	27 Jun 2001
Observation and scoring at 48 hours	28 Jun 2001
Observation and scoring at 72 hours	29 Jun 2001
Audit of raw data and Study Report	13 Jul 2001
Audit findings reported to the Study Director and Management	13 Jul 2001

Process-based monitoring of other common procedures and routine inspection of facilities were also conducted and reported.

C.A. Fuller, M.R.Q.A.
Section Head, Quality Assurance Unit

C.A. Fuller

Date: 18 July 2001

PERSONNEL INVOLVED

Study Director : S J Ridgway, HNC

Report compilation: S J Ridgway, HNC

Scientists : A I MacKay, D Wilding

Quality Assurance : S.M. Loscombe, B.Sc., C.Biol., F.I.B.M.S.
C.A. Fuller, M.R.Q.A.

1. SUMMARY

The objective of the skin irritation test was to assess the potential of Bravo to produce dermal irritation in the rabbit as stipulated in BS EN ISO 10993-10:1996

The results did not indicate a potential of Bravo to cause skin irritation in the rabbit.

2. INTRODUCTION

This part of ISO 10993 assesses possible contact hazards from device-released chemicals that may produce skin and mucosal irritation, eye irritation and delayed contact sensitisation.

The method uses polar and non-polar solvents to obtain extracts that are administered directly to the skin using patches.

Following discussion with the sponsor and consideration of the site of contact of the device in use an acute (single application) of patches was made. The site of application was assessed for 72 hours after the application.

At the request of the sponsor the study was conducted in compliance with the principles of Good Laboratory Practice as set forth in "The Good Laboratory Practice Regulations 1999".

Study timing : Testing commenced: 11th June 2001.

Testing completed : 29th June 2001

Data storage : Raw data, relevant documentation and a copy of the report are archived at Wickham Laboratories Limited. Remaining test articles will be disposed three months after the issue of the final report, unless otherwise directed by the Sponsor.

3. EXPERIMENTAL PROCEDURES

3.1. Materials

3.1.1. Test Item Characterization and Storage

Identity : BRAVO
Container : 1 roll
Sterility status : Non-sterile
Date received : 17th April 2001

On receipt the article was labelled with the unique laboratory reference LR-00190405 and stored at room temperature until required for testing. The identity, strength, purity and stability of the test item was the responsibility of the Sponsor.

3.1.2. Test Material Preparation

BS EN ISO 10993-10:1996 states that if the thickness of the material is less than 0.5 mm use a portion with a total surface area equivalent to 120 cm². Extract this in 20 ml of the extraction medium (this is a 6:1 surface area to volume extraction ratio).

The product is not sterile in-use so there was no requirement to sterilise prior to testing. Using aseptic technique, a test portion of the device measuring 6cm x 10cm was extracted in 20 ml of solvent. Calculations took into account all major surfaces but not the edges or any porous nature of the test material. The test portion, unfragmented, plus extractant and the controls were placed in an incubator for extraction at 37.5 38.0°C for 72 ±1 hours.

3.1.3. Controls

Polar solvent : Sodium chloride 0.9% w/v BP
Non-polar solvent : Sesame oil USP.

For each extractant a control bottle was prepared with a similar volume of extractant but no test material.

3.1.4. Test system

Four New Zealand White rabbits weighing over 2 kg with healthy intact skin, identified by unique ear transponders and caged individually were required for the study.

Sixteen suitable male rabbits were obtained from Charles River UK and allowed to acclimatise for at least seven days. Three days prior to the test the fur on the dorsal surface of the rabbits was clipped and the skin checked. Only rabbits with clear unblemished skin were selected for use on the day of administration: CR017 (3.005kg), CR019 (2.859kg), CR021 (3.087kg) and CR024 (3.127kg).

Details of all rabbit husbandry procedures are given in the appropriate Standard Operating Procedure (MT003).

All rabbits in the colony were individually housed in aluminium cages arranged in racks. Removal from the cage occurred only during test procedures and during routine observations and cleaning. Animal holding rooms and test rooms were maintained at 18±2°C.

All rabbits were allowed free access to water taken from the mains supply and provided in bottles with stainless steel sipper tubes. Rabbits were maintained on a daily ration of standard rabbit maintenance diet (RABMA) presented ad libitum in stainless steel hoppers.

3.2. Methods

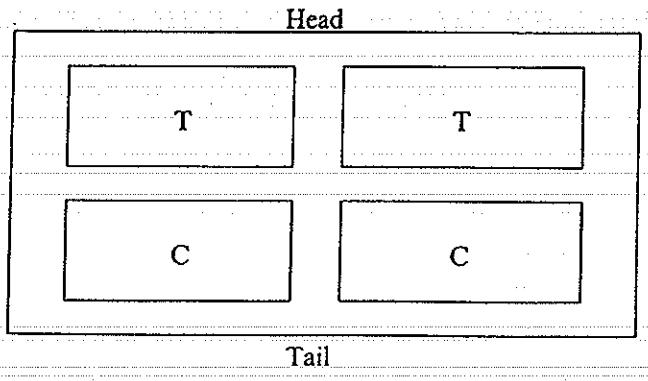
3.2.1. Test Procedure

As very slight signs of irritancy were observed during the Cytotoxity test for this product, precautionary measures were taken. Instead of subjecting all four rabbits to the appropriate extracts, one rabbit (CR017) was allocated to a pilot test using Sodium Chloride for Injection extract and blank. When no reaction was observed, the study was completed using an additional three rabbits. Two rabbits were thus allocated to the polar extract and blank (Sodium chloride 0.9% w/v BP) and two to the non polar extract and blank (Sesame oil USP).

The test procedure was conducted in accordance with BS EN ISO 10993-10:1996 section 5.2. The rabbits were weighed and the weights recorded on the raw data sheet. On the day of the test the fur of the dorsal surface either side of the spine was clipped again.

The appropriate extracts and controls were applied to 25 x 25 mm four-ply gauze patches (0.5 ml extract material per patch). The test (T) and control (C) patches were applied to the dorsal surface of each rabbit as shown in Figure 1.

Figure 1. Arrangement of patches on clipped region



The patches were covered with a semi-occlusive bandage for 4 hours. At the end of this time the dressing was removed and the site of each patch marked. Any remaining test extract was removed by washing with warm water and the area carefully dried.

The appearance of each application site was recorded at 1, 24, 48 and 72 hours after the final removal of the patches.

Any reaction was scored according to the numerical scale in the table below and recorded.

Table 1: Evaluation of Skin Reactions

Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Well defined 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 exposure area)	4

The irritation scores for both erythema and oedema at each time specified were added together. The specified times were at 24, 48 and 72 hours following removal of patches. The score due to the extractant control was subtracted from the test total; this total test was divided by the total number of observations (6) to obtain the irritation score per animal.

The Irritation Scores of each animal were added and divided by the total number of animals, this value was the Cumulative Irritation Index. This value was compared to those in the table 2 and an appropriate response category assigned.

Table 2 Irritation Response Categories in the Rabbits

Response Category	Mean Score
Negligible	0 to 0.4
Slight	0.5 to 1.9
Moderate	2.0 to 4.9
Severe	5.0 to 8.0

4. RESULTS

No adverse clinical effects were observed.

Erythema and oedema were not observed at any stage for rabbits receiving the polar extracts.

A well-defined erythema but no oedema were observed at the non-polar extract sites 24 hours after removal of the patches for rabbit CR024. The sites were warm to the touch across the dorsal surface. The response was the same for the test and control. See Appendix 1. for individual findings.

Table 3. Irritation score for each rabbit

		Irritation Score	
	Rabbit Number	Test	Control
Polar solvent	CR-017	0	0
	CR-019	0	0
Non-polar solvent	CR-021	2	0
	CR-024	8	8

The Cumulative Irritation Index was calculated as 0.33 which falls into the negligible response category.

5. CONCLUSIONS

BRAVO fabric did not cause any irritation in rabbits under the test conditions employed.

6. REFERENCES

6.1. Wickham Laboratories Standard Operating Procedures:

MT003 - Day to Day Care and Welfare of Rabbits
MT005 - Evaluation of Medical Devices for Biological Hazards, Skin Irritancy Test

6.2. BS EN ISO 10993-10:1996 Biological evaluation of medical devices. Tests for irritation and sensitization

Appendix 1 - Individual Rabbit Results

Table 4. Skin irritation scores – CR017 - Polar solvent

Time of scoring	Erythema				Oedema			
	Test		Control		Test		Control	
	L	R	L	R	L	R	L	R
24 hours	0	0	0	0	0	0	0	0
48 hours	0	0	0	0	0	0	0	0
72 hours	0	0	0	0	0	0	0	0

Table 5. Skin irritation scores – CR019 - Polar solvent

Time of scoring	Erythema				Oedema			
	Test		Control		Test		Control	
	L	R	L	R	L	R	L	R
24 hours	0	0	0	0	0	0	0	0
48 hours	0	0	0	0	0	0	0	0
72 hours	0	0	0	0	0	0	0	0

Table 6. Skin irritation scores – CR021 – Non-Polar solvent

Time of scoring	Erythema				Oedema			
	Test		Control		Test		Control	
	L	R	L	R	L	R	L	R
24 hours	1	1	0	0	0	0	0	0
48 hours	0	0	0	0	0	0	0	0
72 hours	0	0	0	0	0	0	0	0

Table 7. Skin irritation scores – CR024 – Non-Polar solvent

Time of scoring	Erythema				Oedema			
	Test		Control		Test		Control	
	L	R	L	R	L	R	L	R
24 hours	2	2	2	2	0	0	0	0
48 hours	1	1	1	1	0	0	0	0
72 hours	1	1	1	1	0	0	0	0