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ELENCO DEI PINCIPALI STUDI SCIENTIFICI EFFETTUATI

1 Norma AFNOR NF T 72-281

Metodo di disinfezione delle superfici per via aerea. Determinazione dell'attività batterica e sporicida secondo la norma AFNOR NF T 72-281 (settembre 1986)

Institute de Recherche Microbiologique (Mitry-Mory.France) / Dr.P.Strohl y A.Carre.- 22 aprile 2008

Documenti :

1 F66 SR attività battericida fungicida e sporicida secondo la norma AFNOR NF T 72-281 franc

1a F66 SR attività battericida fungicida e sporicida secondo la norma AFNOR NF T 72-281 esp

2 Norma UNE-EN 1276 & UNE-EN 1650

Test con sospensione per la valutazione della attività battericida e fungicida.

Laboratorio Biotecnal (Barcelona) / C.Ventura.10 ottobre 2007.

Documenti :

2 F-66 attività battericida secondo la norma UNE-EN 1276

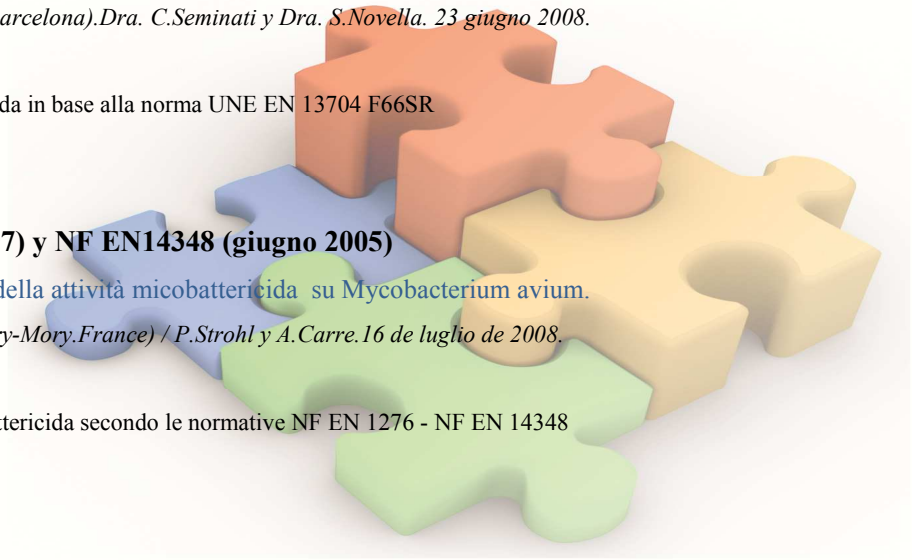
3 Norma UNE-EN 13704

Determinazione dell'attività sporicida dei disinfettanti chimici.

LDG Laboratorio de Diagnóstico General (Barcelona).Dra. C.Seminati y Dra. S.Novella. 23 giugno 2008.

Documenti :

3 F66 SR determinazione dell'attività sporicida in base alla norma UNE EN 13704 F66SR



4 Norma NF EN 1276 (ottobre 1997) y NF EN14348 (giugno 2005)

Test con sospensione per la valutazione della attività micobattericida su Mycobacterium avium.

Institute de Recherche Microbiologique (Mitry-Mory.France) / P.Strohl y A.Carre.16 de luglio de 2008.

Documenti :

4 F66 SR test sospensione - l'attività micobattericida secondo le normative NF EN 1276 - NF EN 14348

5 Norma UNE-EN 1276

Test su batteri multiresistenti al disinfettante di superfici per via aerea

Hospital Clínico San Carlos (Madrid) / Servicio de Medicina Preventiva.29 de octubre de 2008.

Documenti :

5 F66 SR determinazione dell'attivita battericida su betteri multiresistenti

6 UNE-EN 1276 Bactericida frente a cepas de interés alimentario.

Determinazione dell'attività battericida del prodotto in condizioni generali di utilizzo (condizione di sporco) in merito alla Shigella sonnei, Salmonella typhimurium e Listeria monocytogenes.

Departament de Ciència Animal i dels aliments. Universitat Autónoma de Barcelona. Dr.J.J Rodríguez Jérez. Noviembre 2008.

Documenti :

6 F66 SR attività battericida in condizioni di utilizzo su parti sporche secondo la norma UNE-EN-1276

7 Toxicidad Oral Aguda

Studio della tossicità acuta per assunzionein via orale - coi topi.

RCC CIDA. (Barcelona) / M.López y A.Casadesús. 9 mayo de 2008.

Documenti :

7 F66 SR tossicità acuta per assunzione in via orale - studio sui topi

8 I rritación Dérmica

Studio di irritazione primaria nei conigli

RCC CIDA (Barcelona) / M.López y A.Casadesús. 9 junio de 2008.

Documenti :

9 Irritación Ocular

Studio di irritazione oculare primaria nei conigli

RCC CIDA (Barcelona) / M.López y A.Casadesús. 9 mayo de 2008

Documenti :

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N° TVA intracommunautaire :
FR 423 514 221 83

RAPPORT D'ESSAI

N° 218/0408

DELIVRE A : **JOSE COLLADO S.A.**
Espacialidad en Profilaxis
Costa Rica, 35
08027 BARCELONA

PRODUIT : **F66 SR**

APPAREIL : **AEROTURBEX**

DEMANDE D'ESSAI DU : 22 avril 2008

RESULTATS ISSUS DES RAPPORTS : 463/1207 – 146/0208-2

ESSAI : Procédés de désinfection des surfaces par voie aérienne.
Détermination de l'activité bactéricide et sporicide selon la méthodologie de la norme **AFNOR NF T 72-281** (septembre 1986).

Essai sur les souches *Enterococcus hirae* CIP 58.55 et les spores de *Bacillus subtilis* CIP 52 62

Ce rapport comporte 6 pages.

Il ne concerne que le produit soumis à l'essai.

Date d'émission : 29 avril 2008

Amandine CARRE
Microbiologiste
Responsable des essais

Philippe STROHL
Docteur vétérinaire
Directeur Scientifique

I - IDENTIFICATION COMPLETE DE L'ECHANTILLON

Procédé : Dispersion hors présence humaine, par un appareil automatique, d'un liquide destiné à la désinfection des surfaces de locaux.

Nom du produit : **F66 SR**

- Numéro de lot : 2611/L1/2007
- Densité : 1,012
- Fabricant : **JOSE COLLADO S.A.**
  Espacialidad en Profilaxis
  Costa Rica, 35
  08027 BARCELONA

Date de réception à l'I.R.M. : 10 décembre 2007

Conditions de stockage : Dans le laboratoire, à température ambiante.

Composition centésimale : non précisée.

Dose d'essai préconisée : 6,6 ml/m^3

Appareil : **AEROTURBEX**

- Fabricant : **JOSE COLLADO S.A.**
  Espacialidad en Profilaxis
  Costa Rica, 35
  08027 BARCELONA

Période d'essai :

- **463/1207** : du 09 janvier 2008 au 12 janvier 2008
- **146/0208-2** : du 26 février 2008 au 29 février 2008

II - CONDITIONS EXPERIMENTALES

Local d'essai : Salle dédiée, dans les locaux de l'I.R.M.

- Description : Sol et murs carrelés jusqu'au plafond
  Plafond lisse et revêtu d'une peinture vinylique lessivable.
  Une porte et une fenêtre vitrées.
- Volume : 5,75 X 5,94 X 2,48 = 84,7 m^3
- Rapport : $\frac{\text{Surface}}{\text{Volume}} = \frac{125,4}{84,7} = 1,5$

Souches testées :

- **463/1207** : *Enterococcus hirae* CIP 58.55
- **146/0208-2** : *Bacillus subtilis* CIP 52 62

Conditions thermohygrométriques :

			463/1207	**146/0208-2**
• température :	- début	:	21,0°C	21,2°C
	- fin	:	15,0°C	15,5°C
• humidité relative :	- début	:	56%	59%
	- fin	:	99%	99,9%

Supports : Verres de montre en verre de chimie, diamètre = 40 mm.

- Hauteur d'exposition des supports : 80 cm
- Distance par rapport à la source : 2,10 m

Références et nature des membranes utilisées :

- SARTORIUS 13 906 47 ACN
  nitrate de cellulose, porosité 0,45 µm, blanches, quadrillées
- SARTORIUS 13 006 47 ACN
  nitrate de cellulose, porosité 0,45 µm, noires , quadrillées

Utilisation d'un vibreur ultrasonique : non

Temps de diffusion du produit :

- **463/1207** : 16 minutes
- **146/0208-2** : 16 minutes

Temps d'exposition des supports de la fin de la diffusion jusqu'à leur retrait du local : 3 heures

Quantité diffusée :

- **463/1207** : 588 grammes
  Représente une diffusion réelle de 6,86 ml/m^3
- **146/0208-2** : 604,5 grammes
  Représente une diffusion réelle de 7,05 ml/m^3

III - VALIDITE DES ESSAIS PRELIMINAIRES

Souche, collection d'origine et numéro dans la collection	Validité des essais préliminaires	
463/1207		
Enterococcus hirae CIP 58.55	N1 = 104 N2 = 90 N1 = 104	n1 = 103 n2 = 79 n3 = 107
146/0208-2		
Bacillus subtilis CIP 52 62	N1 = 41,5 N2 = 39,5 N1 = 41,5	n1 = 32,75 n2 = 28,5 n3 = 23

N1 = dénombrement de la suspension de microorganismes (méthode par inclusion en milieu gélosé).
N2 = dénombrement de la suspension de microorganismes (méthode par filtration sur membrane).

n1 = validation pour le liquide de recueil en inclusion.
n2 = validation pour le liquide de recueil sur membrane.
n3 = validation du support en inclusion.

Pour chaque souche testée la méthode est validée si :

- **n1 et n3 sont peu différents de N1**
- **n2 est peu différent de N2**

Les conditions expérimentales décrites sont donc applicables lors de l'essai proprement dit.

Les essais préliminaires et les essais proprement dits ont été conduits en parallèle.

IV - RESULTATS DES ESSAIS

Tableau des résultats des essais pour déterminer l'activité désinfectante du couple appareil / produit : **AEROTURBEX / F66 SR**, selon la méthodologie de la norme AFNOR NF T 72 281 (septembre 1986).

Souche, collection d'origine et numéro dans la collection	Dénombrements des microorganismes récupérés	Dénombrements essais			Dénombrements témoins	
		E1	E2	E3	T1	T2
463/1207						
Enterococcus hirae CIP 58.55	1 ml dilution 10^{-3}				63	57
	1 ml dilution 10^{-2}	0	0	0	+	+
	1 ml dilution 10^{-1}	0	0	0		
	1 ml erlen*	0	0	0		
	97 ml	0	0	0		
	porte-microorganismes	0	0	0		
146/0208-2						
Bacillus subtilis CIP 52 62	1 ml dilution 10^{-3}				100,5	92
	1 ml dilution 10^{-2}	0	0	0	+	+
	1 ml dilution 10^{-1}	0	0	0		
	1 ml erlen*	0	0	0		
	97 ml	2	2	5		
	porte-microorganismes	0	0	0		

* Le support est placé dans 100 ml de liquide de récupération, 1 ml de ce liquide est prélevé en duplicata, et 1 ml supplémentaire est utilisé pour les dilutions 10^{-1} à 10^{-3}.

Calcul de la réduction logarithmique du couple appareil / produit : **AEROTURBEX / F66 SR**, selon la méthodologie de la norme AFNOR NF T 72 281 (septembre 1986).

Souche, collection d'origine et numéro dans la collection	T = Dénombrement des porte-germes **témoins** (moyenne de 2 supports)	E = Dénombrement des porte-germes **essais** (moyenne de 3 supports)	d = taux de réduction = activité désinfectante
Bactéricidie – 463/1207			
Enterococcus hirae CIP 58.55	T = $6,0 \times 10^6$	E = 0	d = 6,8 log
Sporicidie – 146/0208-2			
Bacillus subtilis CIP 52 62	T = $9,6 \times 10^6$	E = $3,1 \times 10^5$	d = 6,5 log
d = log T – log E			

Taux de réduction logarithmique minimum exigé pour satisfaire à la norme AFNOR NF T 72-281 :

Activité bactéricide ≥ 5 log

Activité sporicide ≥ 3 log

V - CONCLUSION

L'ensemble des essais réalisés montrent que le couple appareil / produit : **AEROTURBEX / F66 SR** présente par voie aérienne, pour 3 heures de temps de contact après le temps de diffusion du produit, selon la méthodologie de la norme AFNOR NF T 72-281 (septembre 1986), des activités :

- bactéricide sur *Enterococcus hirae* CIP 58.55 pour une dose de 6,86 ml/m^3,

- sporicide sur les spores de *Bacillus subtilis* CIP 52 62 pour une dose de 7,05 ml/m^3

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INFORME DE ENSAYO

N° 218/0408/M-1

ENTREGADO A: **JOSÉ COLLADO, S.A.**
C/ Costa Rica nº 35 local comercial nº 1
08027 BARCELONA (ESPAÑA)

PRODUCTO: **F66 SR**

APARATO: **AEROTURBEX**

SOLICITUD DE ENSAYO: 22 de abril de 2008

ANÁLISIS REFERENCIA: 463/1207, 146/0208-2

ENSAYO: Método de desinfección de superficies por vía aérea.
Determinación de la actividad bactericida, fungicida y esporicida según metodología de la norma **AFNOR NF T 72-281** (septiembre 1986).

Ensayo con *Enterococcus hirae* CIP 58.55 y las esporas de *Bacillus subtilis* CIP 52 62

Este informe consta de 6 páginas.

Unicamente afecta al producto sometido a ensayo.

Fecha de emisión: 29 de abril del 2.008.

Fecha de reemision : 11 de junio del 2008

Sólo la versión francesa da fe

Amandine Carre
Microbióloga
Responsable de los Ensayos

Philippe Strohl
Doctor Veterinario
Director científico

I. IDENTIFICACIÓN COMPLETA DE LA MUESTRA

Procedimiento : Dispersión, en ausencia de personas, mediante un aparato automático, de un liquido destinado a la desinfección de superficies de locales

Nombre del producto : **F66 SR**

- Número de lote : 2611/L1/2007
- Densidad : 1,012

- Fabricante : **JOSÉ COLLADO S.A.**
  C/ Costa Rica nº 35 local comercial nº 1
  08027 BARCELONA (ESPAÑA)

Condiciones de almacenamiento : En el laboratorio, a temperatura ambiente.

Composición centesimal : no precisada.

Dosis de producto recomendada : 6 ml/m^3.

Aparato : **AEROTURBEX**

- Fabricante : **JOSÉ COLLADO S.A.**
  C/ Costa Rica nº 35 local comercial nº 1
  08027 BARCELONA (ESPAÑA)

Periodo de ensayo.:

- **463/1207** : Del 9 de enero al 12 de enero de 2008
- **146/0208-2** : Del 26 de febrero al 29 de febrero de 2008.

II. CONDICIONES EXPERIMENTALES

Local de ensayo: Sala específica, en las instalaciones de I.R.M.

* Descripción: - Suelos y paredes embaldosadas.
- Techo liso y cubierto de una pintura vinílica lavable.
- Una puerta y una ventana acristaladas.

* Volumen: $5,75 \times 5,94 \times 2,48 = 84,7\ m^3$

* Relación : $\frac{\text{Superficie}}{\text{Volumen}} = \frac{125,4}{84,7} = 1,5$

Cepas de ensayo:

- **463/1207** : *Enterococcus hirae* CIP 58. 55
- **146/0208-2** : *Bacillus subtillis* CIP 52 62

Condiciones termo-higrométricas:

		463/1207	**146/0208-2**
• Temperatura:	- inicio :	21,0 °C	21,2 °C
	- final	15,0 °C	15,5 °C
• Humedad relativa:	- inicio	56%	59%
	- final	99 %	99,9%

Soportes: vidrios de reloj de cristal químico, de 40 mm. de diámetro.

* Altura de exposición de los soportes : 80 cm.
* Distancia en relación a la fuente : 2,10 m.

Referencia y naturaleza de la membrana utilizada

* SARTORIUS 13 906 47 ACN, nitrato de celulosa, porosidad 0,45 µm, blancas y cuadriculadas

* SARTORIUS 13 006 47 ACN, nitrato de celulosa, porosidad 0,45 µm, negras y cuadriculadas

Utilización de un ultrasonidos: no.

Tiempo de difusión del producto:

- **463/1207** : 16 minutos.
- **146/0208-2** : 16 minutos.

Tiempo de exposición de los soportes desde el final de la emisión hasta su retirada del local: 3 horas.

Cantidad difundida:

- **463/1207** : 588 gr.
  Representa una difusión real de 6,86 ml/m^3
- **146 / 0208-2** : 604,5 gr.
  Representa una difusión real de 7,05 ml/m^3

III. VALIDACION DE LOS ENSAYOS PRELIMINARES

Cepa, colección de origen y numero de colección	Validación de los ensayos preliminares	
463/1207		
Enterococcus hirae CIP 58.55	N1 = 104 N2 = 90 N1 = 104	n1 = 103 n2 = 79 n3 = 107
146/0208-2		
Bacillus subtilis CIP 52 62	N1 = 41,5 N2 = 39,5 N1 = 41,5	n1 = 32,75 n2 = 28,5 n3 = 23

N1 = recuento de la suspensión de microorganismos (método de inclusión en medio cultivo sólido)
N2 = recuento de la suspensión de microorganismos (método por filtración sobre membrana).
n1 = validación del liquido de recuento en inclusión
n2 = validación del liquido de recuento sobre membrana.
n3 = validación del soporte en inclusión.

Para cada cepa estudiada el método es válido sí:

- **n1 y n3 se diferencian poco de N1**
- **n2 se diferencia poco de N2**

Las condiciones experimentales descritas son aplicables al ensayo propiamente dichas.

Los ensayos preliminares y los finales se hacen en paralelo.

IV. RESULTADOS DEL ENSAYO

Tabla de resultados del ensayo para determinar la actividad del desinfectante del binomio aparato / producto **AEROTURBEX / F66 SR** según la norma AFNOR NF T 72 – 281 (septiembre 1986).

Cepa, colección de origen y numero de colección	Recuento de los microorganismos recuperados	Recuento del ensayo			Recuento del testigo	
		E 1	E 2	E 3	T 1	T 2
462/1207						
Enterococcus hirae CIP 58.55	1 ml dilución 10^{-3}				63	57
	1 ml dilución 10^{-2}	0	0	0	+	+
	1 ml dilución 10^{-1}	0	0	0		
	1 ml erlen*	0	0	0		
	97 ml	0	0	0		
	Portagérmemes	0	0	0		
146/0208-2						
Bacillus subtilis CIP 52 62	1 ml dilución 10^{-3}				100,5	92
	1 ml dilución 10^{-2}	0	0	0	+	+
	1 ml dilución 10^{-1}	0	0	0		
	1 ml erlen*	0	0	0		
	97 ml	2	2	5		
	Portagérmemes	0	0	0		

* El soporte se sumerge en 100 ml. de recuperación, 1 ml de este líquido se siembra por duplicado y otro ml. se utiliza para realizar las diluciones 10^{-1} a 10^{-3} .

Cálculo de las reducciones logarítmicas del binomio aparato / producto **AEROTURBEX / F66 SR** según la norma AFNOR NF T 72 – 281 (septiembre 1986).

Cepa, colección de origen y numero de colección	T = inoculo testigo (media de dos portagérmenes) UFC/soporte	E = contage de portagérmenes Del ensayo (Media de 3 soportes) UFC/soporte	d = tasa de reducción = actividad del desinfectante
Bactericida - 463/1207			
Enterococcus hirae CIP 58 55	T = $6,0 \times 10^6$	E = 0	d = 6,8 log
Esporcida – 146/0208-2			
Bacillus subtilis CIP 52 62	T = $9,6 \times 10^6$	E = $3,1 \times 10^5$	d = 6,5 log

Tasa de reducción mínima exigida para satisfacer la norma AFNOR NF T 72-281:

- **Actividad bactericida ≥ 5**

- **Actividad esporicida ≥ 3**

VI. CONCLUSIÓN

El binomio aparato / producto **AEROTURBEX / F66 SR** presenta por vía aérea, con 3 horas de tiempo de contacto después de la difusión del producto, según la norma AFNOR NF T 72-281 (septiembre 1986) las actividades:

- Bactericida frente a *Enterococcus hirae* CIP 58.55 a una dosis de 6,86 ml/m^3,

- Esporicida frente a esporas de *Bacillus subtilis* CIP 52 62 a una dosis de 7.05 ml/m^3

INFORME DE ENSAYO

Nº INFORME **O-650.028(GU)**

Identificación del cliente:

Nombre:	JOSE COLLADO SA
Dirección:	C/ COSTA RICA Nº 35, LOCAL COMERCIAL Nº 1
Localidad:	BARCELONA
Provincia:	BARCELONA
Código postal:	08027
A / A:	SRA. Mª JOSÉ COLLADO
Código cliente:	377

Control fechas:

Recepción:	03-10-2007
Inicio:	05-10-2007
Finalización:	10-10-2007

Identificación de la muestra:

Producto : F66 SR

Marca:	JOSE COLLADO
Lote:	-
Lugar recogida:	-
Fecha toma muestra:	03-10-2007
Temperatura recepción laboratorio:	AMBIENTE

INFORME DE ENSAYO

ACTIVIDAD BACTERICIDA (UNE-EN 1276):

Determinación de la actividad bactericida del producto en condiciones de utilización generales (Condiciones limpias)

a) Identificación del laboratorio de ensayo: Biotecnal, S.A.

b) Identificación de la muestra:

Número de referencia interna:	O-650.028(GU)
Nombre del producto:	F66 SR
Número del lote:	/
Fabricante:	JOSE COLLADO,S.A.
Fecha de entrega:	03-10-07
Condiciones de almacenamiento:	Temperatura ambiente
Diluyente del producto, recomendado por el fabricante:	Agua potable
Sustancia(s) activa(s) y su(s) concentración(es):	Peróxido de hidrógeno (8%),Alcohol isopropilico (9,99%)

c)Método de ensayo y su validación:

Método:	Dilución-Neutralización
Neutralizador:	Caldo neutralizador D/E

d)Condiciones experimentales:

Período del análisis:	05-10-07 al 10-10-07
Diluyente del producto utilizado durante el ensayo:	Agua dura estéril
Concentraciones de ensayo del producto:	20%, 40% y 80% (v/v)
Temperatura del ensayo:	23 $\pm$ 1 ºC
Aspecto del producto y de las diluciones del mismo:	Soluciones incoloras
Sustancia interferente:	Solución acuosa de albúmina bovina 3 g/l
Estabilidad de la mezcla (sustancia interferente y producto diluidos en agua dura):	Ausencia de precipitado durante el ensayo
Tiempo de contacto:	5 minutos $\pm$ 10 segundos.
Temperatura de incubación:	37 $\pm$ 1 ºC
Identificación de las cepas bactericidas utilizadas:	*Pseudomonas aeruginosa CECT-116* *Escherichia coli* CECT-405 *Staphylococcus aureus* CECT-59 *Enterococcus hirae* CECT-4081

e) Resultados del ensayo:
(Véase tabla 1,2,3,4)

f) Conclusión:
De acuerdo con la Norma EN 1276 (1997) el producto F66 SR (O-650.028(GU)*)* cuando está diluido al 40 % (v/v) en agua dura, posee actividad bactericida después de 5 minutos a 23 ºC en condiciones sucias (solución acuosa de albúmina bovina 3 g/l) para las cepas de referencia *Pseudomonas aeruginosa* CECT - 116, *Escherichia coli CECT-405, Staphylococcus aureus* CECT-59 *y Enterococcus hirae* CECT-4081

INFORME DE ENSAYO

ACTIVIDAD FUNGICIDA (UNE-EN 1650):

Determinación de la actividad fungicida del producto en condiciones de utilización generales (Condiciones limpias)

a) Identificación del laboratorio de ensayo:	Biotecnal, S.A.
b) Identificación de la muestra:	
Número de referencia interna:	O-650.028(GU)
Nombre del producto:	F66 SR
Número del lote:	-
Fabricante:	JOSE COLLADO S.A.
Fecha de entrega:	03-10-07
Condiciones de almacenamiento:	Temperatura ambiente
Diluyente del producto, recomendado por el fabricante:	Agua potable
Sustancia(s) activa(s) y su(s) concentración(es):	Peróxido de hidrógeno (8%), Alcohol isopropilico (9,99%)
c) Método de ensayo y su validación:	
Método:	Dilución-Neutralización
Neutralizador:	Caldo Neutralizador D/E
d) Condiciones experimentales:	
Período del análisis:	05-10-07 al 10-10-07
Diluyente del producto utilizado durante el ensayo:	Agua dura estéril
Concentraciones de ensayo del producto:	20%,40% y 80% (v/v)
Temperatura del ensayo:	23 $\pm$ 1 ºC
Aspecto del producto y de las diluciones del mismo:	Soluciones incoloras
Sustancia interferente:	Solución acuosa de albúmina bovina 3 g/l.
Estabilidad de la mezcla (sustancia interferente y producto diluidos en agua dura):	Ausencia de precipitado durante el ensayo
Tiempo de contacto:	15 minutos $\pm$ 10 segundos.
Temperatura de incubación:	30 $\pm$ 1 ºC
Identificación de las cepas funguicidas utilizadas:	*Candida Albicans* CECT-1394 *Aspergillus Niger* CECT-2574

e) Resultados del ensayo:
(véase tablas B5,B6)

f) Conclusión:
De acuerdo con la Norma EN 1650 (1997) el producto F66 SR (O-650.028(GU)*)* cuando está diluido al 40 % (v/v) en agua dura, posee actividad fungicida después de 15 minutos a 23 ºC en condiciones sucias (solución acuosa de albúmina bovina 3 g/l) para la cepa de referencia *Aspergillus niger* CECT-2574. Posee actividad fungica después de 15 minutos a 23ºC para la cepa de referencia *Candida Albicans*, cuando está diluido al 80%

INFORME DE ENSAYO

Nº INFORME **O-650.028(GU)**

TABLA B-1

***Pseudomonas aeruginosa* CECT – 116 (MR-84)**				
Ensayo de validación:				
Suspensión bacteriana: Nv	**Condiciones experimentales: A**		**Control de la toxicidad: B**	**Control del método: C**
Vc: 209-199 **Nv:2,0 x10^3**	Vc:196-199 **A:2,0 x10^2**		Vc:203-201 **B:2,0 x10^2**	Vc.198-190 **C:1,9x10^2**
Suspensión bacteriana de ensayo: N	**Procedimiento de ensayo**			
		Ni 20%	**Nd 40%**	**Ns 80%**
10-^{6}:244-235 10-^{7}:25-25 **N:2,4x10^8**	Vc Na **R**	63-88 7,6x10^3 **3,2x10^3**	7-4 < 1,5x10^2 **> 10^5**	0-0 < 1,5x10^2 **> 10^5**

INFORME DE ENSAYO

Nº INFORME **O-650.028(GU)**

biotecnal TECNOBROMATOLOGIA INDUSTRIAL

TABLA B-2

***Escherichia coli* CECT – 405 (MR-22)**				
Ensayo de validación:				
Suspensión bacteriana: Nv	**Condiciones experimentales: A**		**Control de la toxicidad: B**	**Control del método: C**
Vc:176-184 **Nv:1,8x10^3**	Vc:177-170 **A:1,7x10^2**		Vc:169-173 **B:1,7x10^2**	Vc.179-180 **C:1,8x10^2**
Suspensión bacteriana de ensayo: N	**Procedimiento de ensayo**			
		Ni 20%	**Nd 40%**	**Ns 80%**
10-^{6}:210-214	Vc	95-80	8-8	0-0
10-^{7}:22-22	Na	8,8x10^3	< 1,5x10^2	< 1,5x10^2
N:2,1x10^8	**R**	**2,4 x 10^3**	**> 10^5**	**> 10^5**

INFORME DE ENSAYO

Nº INFORME **O-650.028(GU)**

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TECNOBROMATOLOGIA INDUSTRIAL

TABLA B-3

<table>
<tr><th colspan="6">Staphylococcus aureus CECT – 59 (MR-31)</th></tr>
<tr><th colspan="6">Ensayo de validación:</th></tr>
<tr><th>Suspensión bacteriana:
Nv</th><th colspan="2">Condiciones experimentales:
A</th><th>Control de la toxicidad:
B</th><th colspan="2">Control del método:
C</th></tr>
<tr><td>Vc:181-197

Nv:1,9x10^3</td><td colspan="2">Vc:188-179

A:1,8x10^2</td><td>Vc:185-180

B:1,8x10^2</td><td colspan="2">Vc.183-189

C:1,9x10^2</td></tr>
<tr><td rowspan="2">Suspensión bacteriana de ensayo: N</td><td colspan="5">Procedimiento de ensayo</td></tr>
<tr><td colspan="2">Ni
20%</td><td>Nd
40%</td><td colspan="2">Ns
80%</td></tr>
<tr><td>10-^{6}:179-188

10-^{7}:19-18

N:1,8x10^8</td><td>Vc

Na

R</td><td>102-96

9,9x10^3

1,8x10^3</td><td>10-8

< 1,5x10^2

> 10^5</td><td colspan="2">0-0

< 1,5x10^2

> 10^5</td></tr>
</table>

INFORME DE ENSAYO

Nº INFORME **O-650.028(GU)**

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TABLA B-4

Enterococcus hirae **CECT –4081 (MR-85)**				
Ensayo de validación:				
Suspensión bacteriana: Nv	**Condiciones experimentales: A**		**Control de la toxicidad: B**	**Control del método: C**
Vc:159-172 **Nv:1,7x10^3**	Vc:162-168 **A:1,7x10^2**		Vc:163-163 **B:1,6x10^2**	Vc.170-165 **C:1,7x10^2**
Suspensión bacteriana de ensayo: N	**Procedimiento de ensayo**			
		Ni 20%	**Nd 40%**	**Ns 80%**
10-^{6}: 197-190 10-^{7}:20-21 **N:2,0x10^8**	Vc Na **R**	128-141 1.4x10^4 **1,4 x 10^3**	13-15 < 1,5x10^2 **> 10^5**	0-0 < 1,5x10^2 **> 10^5**

INFORME DE ENSAYO

Nº INFORME **O-650.028(GU)**

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TECNOBROMATOLOGIA INDUSTRIAL

TABLA B-5

Aspergillus niger **CECT – 2574 (MR-69)**				
Ensayo de validación:				
Suspensión fúngica: Nv	**Condiciones experimentales: A**		**Control de la toxicidad: B**	**Control del método: C**
Vc: 86-71 **Nv: 7,9 x 10^2**	Vc: 83-80 **A: 8,2 x 10**		Vc: 79-76 **B: 7,8 x 10**	Vc: 77-82 **C: 8,0 x 10**
Suspensión fúngica de ensayo: N	**Procedimiento de ensayo**			
	Ni 20%		**Nd 40%**	**Ns 80%**
10^{-5} :>150 ->150 10^{-6}: 25-19 **N: 2,2 x 10^7**	Vc Na **R**	32-21 2,7 x 10^3 **8,1 x 10^2**	3-3 < 1,5 x 10^2 **> 10^4**	0-0 < 1,5x10^2 **> 10^4**

INFORME DE ENSAYO

Nº INFORME **O-650.028(GU)**

biotecnal TECNOBROMATOLOGIA INDUSTRIAL

TABLA B-6

***Candida albicans* CECT – 1394 (MR-70)**				
Ensayo de validación:				
Suspensión fúngica: Nv	**Condiciones experimentales: A**		**Control de la toxicidad: B**	**Control del método: C**
Vc: 126-120 **Nv: 1,2 x 10^3**	Vc: 121-119 **A: 1,2 x 10^2**		Vc: 117-123 **B: 1,2 x 10^2**	Vc: 121-121 **C: 1,2 x 10^2**
Suspensión fúngica de ensayo: N	**Procedimiento de ensayo**			
		Ni 20%	**Nd 40%**	**Ns 80%**
10^{-5}: >150->150	Vc	>150 - >150	61-53	0-0
10^{-6}: 44-40	Na	> 1,5 x 10^3	5,7 x 10^2	< 1,5x10^2
N: 4,2 x10^7	**R**	**< 10^4**	**7,4 x 10^3**	**> 10^4**

Sant Quirze del Vallés, 10 de Octubre de 2007

Carisia Ventura i Cebrià
Director Técnico

biotecnal TECNOBROMATOLOGIA INDUSTRIAL

Laboratorio de Diagnóstico General

INFORME DE RESULTADOS

DETERMINACION DE LA ACTIVIDAD ESPORICIDA DE LOS DESINFECTANTES QUIMICOS

(Norma: UNE-EN 13704)

PRODUCTO ESTUDIADO: F66 SR

CÓDIGO DE LA MUESTRA: M-08-118953

FECHA: 23 de Junio de 2008

SOLICITANTE

Mª José Collado

JOSE COLLADO, S.A.

C/ Costa Rica nº 35
Local comercial 1
08027 Barcelona

REALIZACION

LABORATORIO DE DIAGNOSTICO GENERAL
C/ Comte Borrell 111, bajos
08015 - Barcelona

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INDICE

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1. ANTECEDENTES

Esta Norma Europea describe un método de ensayo de suspensión para determinar si un desinfectante químico posee actividad esporicida en las condiciones experimentales definidas en la misma.

Por otra parte especifica un método de ensayo y los requisitos mínimos para verificar la actividad esporicida de productos químicos desinfectantes que forman una preparación homogénea físicamente estable en agua dura.

Esta Norma Europea es aplicable a los productos que se utilizan en productos alimenticios, en la industria, en el hogar y en colectividades, excluyendo las áreas y situaciones en las que la desinfección está médicamente indicada y excluyendo los productos utilizados sobre tejidos vivos, con excepción de los utilizados para la higiene de las manos en las áreas anteriormente consideradas.

Se considera que un producto sometido a ensayo posee actividad esporicida cuando se demuestra una reducción de al menos 10^3 de los recuentos viables.

Simultáneamente se realiza la validación del método tal como se describe en la Norma, para verificar la concentración de la suspensión de esporas, las condiciones experimentales, el control de la toxicidad del neutralizador y el control de dilución-neutralizador.

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2. IDENTIFICACIÓN DE LAS MUESTRAS

La muestra se recibe el día 19 de mayo de 2008 en el Laboratorio de Diagnóstico General debidamente acondicionada en envase cerrado y conteniendo en la etiqueta la siguiente información:

"F66 SR"

Mediante información adjunta se incluyen las características del producto y la petición de ensayo que consiste en la aplicación del producto a tres concentraciones del 0.01, 50 y 100% durante un tiempo de contacto de 60 minutos para la evaluación de la actividad esporicida, según Norma UNE-EN 13704 de los desinfectantes químicos.

La muestra se identifica con el Código de Muestra: M-08-118953.

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3. VALORACIÓN SEGÚN NORMA UNE-EN 13704

DETERMINACIÓN DE LA ACTIVIDAD ESPORICIDA.

Identificación del laboratorio de ensayo	LABORATORIO DE DIAGNOSTICO GENERAL
Identificación de la muestra	
Código de la muestra	M-08-118953
Nombre del producto	F66 SR
Número de lote	150S/LI/2008
Fabricante	JOSE COLLADO S.A.
Fecha de recepción	19/05/08
Condiciones de almacenamiento	4ºC
Producto diluyente recomendado por el fabricante	No indicado
Ingrediente(s) activo(s)	Peroxido de hidrogeno
Método de ensayo y su validación	
Método	Dilución-Neutralización
Neutralizador	-
Condiciones experimentales	
Período de análisis	Del 30.05.08 al 02.06.08
Diluyente del producto utilizado durante el ensayo	Agua dura estéril 300 mg/Kg $CaCO_3$
Concentraciones del producto sometido a ensayo	0.01, 50 y 100%
Aspecto de las diluciones del producto	Solución del producto clara e incolora
Estabilidad de la mezcla (sustancia interfiriente y producto diluidos en agua dura)	Ausencia del precipitado durante el ensayo
Sustancia interfiriente	Albúmina bovina 0.3 g/l
Temperatura de ensayo	20ºC ± 1ºC
Tiempo de contacto	60 minutos
Temperatura de incubación	30 ºC± 1ºC
Identificación de las cepas utilizadas	❑ *Bacillus subtilis* CIP 52.62
Suspensión de esporas	Lote nº 24 CIP 52.62
Origen de las esporas	-
Resultados del ensayo	Ver Tablas en: Pág. 6-7 de 7

Conclusión:
De acuerdo con la Norma UNE-EN 13704: 2002, el producto F66 SR cuando está diluido al 50% en agua dura, **posee actividad esporicida** después de sesenta minutos a 20ºC en condiciones limpias (solución acuosa de 0.3 g/l de albúmina bovina) para la cepa de referencia *Bacillus subtilis* CIP 52.62.

Barcelona, 18 de junio de 2008

Dra. Chiara Seminati
Responsable de sección

Dra. Sònia Novella
Directora Técnica

LDG es laboratorio de ensayo. Inscrito en el registro de laboratorios Agroalimentarios, Laboratori Agrari de Cabrils, Generalitat de Catalunya. Laboratorio acreditado para la realización de ensayos en productos de alimentación animal, humana y análisis de residuos en canales de animales y en órganos diana. Acreditado como laboratorio de Salud Pública del Departament de Sanitat i Seguretat Social. LDG es entidad de Certificación y Control de Calidad por el Server de Denominacions y Marques. Entidad Colaboradora de l'Agencia Catalana del Aigua, Departamento de Medio Ambiente, Generalitat de Catalunya. Capacitado por el ministerio de Agricultura, Pesca y Alimentación y la Subdirección General de Sanidad Animal para la realización de análisis de sustancias medicamentosas veterinarias. Habilitado por la Subdirección General de Sanidad Exterior y Veterinaria puestos de inspección fronteriza. LDGes laboratorio autorizado para la formación de manipuladores de alimentos según el R.D. 202/2000

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4. RESULTADOS

Organismo de ensayo	Ensayo de validación				Suspensión de esporas de ensayo	Procedimiento de ensayo a la concentración %	
	Suspensión de esporas	Condiciones experimentales	Control de la toxicidad del neutralizador	Control del método de dilución-neutralización			0.01%
Bacillus subtilis CIP 52.62	10^{-1}: 78; 70 Nv: 7.4×10^{2}	10^{-0}: 68; 75 A: 7.2×10^{1}	10^{-0}: 66; 70 B: 6.8×10^{1}	10^{-0}: 71;64 C: 6.8×10^{1}	10^{-4}: 210; 260 N: 2.0×10^{6}	V_c N_a **R**	10^{0}: >300;>300 1.8×10^{4} **$< 10^{3}$**

Organismo de ensayo	Ensayo de validación				Suspensión de esporas de ensayo	Procedimiento de ensayo a la concentración %	
	Suspensión de esporas	Condiciones experimentales	Control de la toxicidad del neutralizador	Control del método de dilución-neutralización			50%
Bacillus subtilis CIP 52.62	10^{-1}: 78; 70 Nv: 7.4×10^{2}	10^{-0}: 68; 75 A: 7.2×10^{1}	10^{-0}: 66; 70 B: 6.8×10^{1}	10^{-0}: 71;64 C: 6.8×10^{1}	10^{-4}: 210; 260 N: 2.0×10^{6}	V_c N_a **R**	10^{0}: 0; 0 $< 1.5 \times 10^{2}$ **$> 10^{3}$**

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Organismo de ensayo	Ensayo de validación				Suspensión de esporas de ensayo	Procedimiento de ensayo a la concentración %	
	Suspensión de esporas	Condiciones experimentales	Control de la toxicidad del neutralizador	Control del método de dilución-neutralización			100%
Bacillus subtilis CIP 52.62	10^{-1}: 78; 70 Nv: $7.4x10^{2}$	10^{-0}: 68; 75 A: $7.2\ x10^{1}$	10^{-0}: 66; 70 B: $6.8\ x10^{1}$	10^{-0}: 71;64 C: $6.8x10^{1}$	10^{-4}: 210; 260 N: $2.0\ x10^{6}$	V_c N_a **R**	10^{0}: 0, 0 $< 1.5\ x10^{2}$ **$> 10^{3}$**

Vc = recuento viable
N = número de cfu/ml de la suspensión de esporas del ensayo
Nv= número de cfu/ml de la suspensión de esporas
R = reducción de la viabilidad
Na= número de cfu/ml en la mezcla de ensayo
A = número de cfu/ml obtenido en el ensayo de validación de las condiciones experimentales
B = número de cfu/ml obtenido en el ensayo de validación de la toxicidad del neutralizador
C = número de cfu/ml obtenido en el ensayo de validación del método de dilución-neutralización

Verificación de la metodología: para cada organismo se verifica que,

N: está comprendido entre $1,5 \times 10^{6}$ cfu/ml y 5×10^{6} cfu/ml
Nv: está comprendido entre 6×10^{2} cfu/ml y $3\ x10^{3}$ cfu/ml
B: es igual o superior a 0,05 veces el valor de N_v
C: es igual o superior a 0,5 veces el valor de B
A: es igual o superior a 0,05 veces el valor de N_v

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Institut de
Recherche
Microbiologique

BOLETIN DE ENSAYO
Nº 146/0208-4/M-1

ENTREGADO A: **JOSÉ COLLADO, S.A.**
C/ Costa Rica nº 35 local comercial nº 1
08027 BARCELONA
ESPAÑA

PRODUCTO: **F66 SR**

SOLICITUD DE ENSAYO: 4 de febrero 2008

ANÁLISIS REFERENCIA: 463/1207

ENSAYO: Ensayo cuantitativo de suspensión para la evaluación de la actividad micobactericida. según normas NF EN 1276 (octubre 1997) y NF EN 14348. (junio 2005).

Método por filtración de membrana.

El Producto se aplica puro.

Ensayo frente *Mycobacterium avium* CIP 105 415

Este informe consta de 5 páginas.
Sólo concierne al producto sometido a ensayo
Fecha de emisión: 16 de Julio 2008
Fecha de reemision : 25 de noviembre 2008
Sólo la versión francesa da fe

Amandine CARRE
Microbióloga
Responsable de los Ensayos

Philippe STROHL
Doctor en Veterinaria
Director Científico

I. IDENTIFICACIÓN COMPLETA DE LA MUESTRA

Nombre del producto: **F66 SR**

* número de lote: 2611/L1/2007

* fabricante: **JOSÉ COLLADO, S.A.**
C/ Costa Rica nº 35 local comercial nº 1
08027-BARCELONA
ESPAÑA

Fecha de recepción en I.R.M.: 10 de diciembre 2007.

Condiciones de almacenamiento: En la poyata del laboratorio, a temperatura ambiente.

Período de análisis: del 24 junio al 15 de julio 2008

Sustancia(s) activa(s) y concentración(es) : . no precisadas

Aspecto del producto y de sus diluciones : solución límpida incolora.

II. CONDICIONES EXPERIMENTALES

Tiempo de contacto: 60 minutos.

Temperatura de ensayo: 20.± 1 ° C

Diluyente del producto utilizado en los ensayos: agua destilada estéril.

Diluyente del producto recomendado por el fabricante: ninguno.

Cepa estudiadas : (NF EN 14348. (junio 2005))

- *Mycobacterium avium* CIP 105 415 Incubación 21 días a 37 ± 1 °C

Sustancia interferente: condiciones limpias (NF EN 14348. (junio 2005))

- Composición: 0'3 g / l de albúmina bovina al final de la prueba

Estabilidad de la mezcla producto – sustancia interferente : ausencia de precipitación durante el ensayo.

Método de recuento: recuento por siembra en superficie para recuento del inóculo y por filtración sobre membrana para el ensayo de validación y el ensayo propiamente dicho.

III. METODICA PARA LOS ENSAYOS PRELIMINARES

Líquido de lavado de las membranas :

* Composición: agua destilada añadiendo 0,5% (v/v) de tween 80.

* Modo de preparación : Disolución de todos los ingredientes en caliente y esterilización: autoclave a 122ºC durante 15 minutos.

Método de lavado de membranas :

* Número de lavados con el liquido : 3
* Volumen del liquido que se utiliza para cada lavado : 50 ml.

Neutralizante(s) añadido(s) al medio de cultivo y concentración(es): ninguno.

Otros aditivos al medio de cultivo : ninguno

Medios de cultivo selectivos: (norma NF EN 14348. (junio 2005))

* Medio sólido 7H10 MIDELBROOK enriquecido con un 10 % de OADC.

IV. VALIDACIÓN DEL METODO DE FILTRACIÓN SOBRE MENBRANA

Cepa de Micro-organismos	Concentración del producto ensayado	Suspensión bacteriana de ensayo	Resultados a la concentración m % (v/v)			
			Suspensión bacteriana	Condiciones experimentales	Testigo de filtración	Inactivación por filtración
Mycobacterium avium CIP 105 415	80% (v/v)	10^{-6} :>300;>300 10^{-7} :34 ; 34 (N = 3,4 x 10^{8})	80; 74 (Nv = 770)	60; 63 (A = 61,5)	62; 67 (B = 64,5)	59; 70 (C = 64,5)

N = Recuento de UFC./ml. de la suspensión bacteriana de ensayo.
N_v = Recuento de UFC./ml. de la suspensión bacteriana.
A = Recuento de UFC./ml. en el ensayo de validación en condiciones experimentales.
B = Recuento de UFC./ml. en el blanco de la filtración de membrana
C = Recuento de UFC./ml. en el ensayo de validación por la filtración sobre membrana

El método de filtración sobre membrana se valida si:

- N, se encuentra entre 1,5 x 10^{8} UFC/ ml. y 5x 10^{8} UFC/ ml.
- Nv , se encuentra entre 6 x 10^{2} UFC/ ml. y 3 x 10^{3} UFC/ ml.
- A y B son superiores ó iguales a 0,005 N.
- C es superior ó igual a 0,5 B.

El método de filtración sobre membrana queda validado en las condiciones descritas frente a *Mycobacterium avium* CIP 105 415 a una concentración del 80 % (v/v) de **F66 SR.**

V. RESULTADOS DE LOS ENSAYOS

Cepa de Microorganismos	Suspensión bacteriana de ensayo	Resultados a la concentración m % (v/v)			
			m = 80	m = 40	m = 20
Mycobacterium avium CIP 105 415	10^{-6} :>300;>300 10^{-7} :34 ; 34 (N = 3,4 x 10^{8})	Vc Na R	0 ; 0 < 150 **>2,3 x 10^{5}**	>150;>150 >1 500 <2,3 x 10^{4}	>150;>150 >1 500 <2,3 x 10^{4}

N = Recuento de UFC./ml. de la suspensión bacteriana de ensayo.
V_c= Recuento del número de colonias sobre la placa.
N_a= Recuento de UFC / ml. en la mezcla de ensayo.
R = reducción del número de células viables.

Según los criterio de la norma NF EN 1276 (octubre 1997), El producto es bactericida si la concentración estudiada da lugar a una reducción de cómo mínimo **10^5** de células viables.

VI. CONCLUSIÓN

Según los métodos de las normas NF EN 1276 (octubre 1997) y NF EN 14348 (junio 2005), el producto **F66 SR** presenta una actividad micobactericida frente a *Mycobacterium avium* CIP 105 415 a la concentración del 80 % (v/v), en 60 minutos de contacto y a 20°C, en presencia de 0,3 g/l albúmina bovina (condiciones limpias).

INFORME DEL ENSAYO DE DETERMINACIÓN DE LA ACTIVIDAD BACTERICIDA FRENTE A BACTERIAS MULTIRRESISTENTES DEL DESINFECTANTE DE SUPERFICIES POR VÍA AÉREA F66 SR.

Identificación de la muestra

Nombre del producto .....**F66 SR**
Número de lote 2107/L1/2008
Fabricante .....JOSÉ COLLADO, S.A.
Fecha de entrega 29/07/2008
Diluyente que el fabricante recomienda .....No indicado
Sustancia activa y su concentración (en 100 ml).....8% Peróxido de hidrógeno

Método del ensayo y su validación basado en UNE EN 1276

Método.....Filtración sobre membrana
Diluyente.....Agua estéril destilada.

Condiciones experimentales

Período de análisis.....Agosto 2008-Octubre 2008
Diluyente del producto utilizado durante el ensayo .....Agua estéril destilada
Concentraciones de ensayo a someter a ensayo .....20%; 40%; 80% (v/v).
Tiempo de contacto .....1 y 5 minutos.
Temperatura de ensayo..... 25 ± 1ºC
Sustancia interfiriente (condiciones limpias) .....Solución albúmina bovina 0.3 g/l.
Estabilidad de la muestra..... Ausencia de precipitado
Temperatura de incubación.....36ºC ± 1ºC.
Identificación y resistencias de las cepas bacterianas utilizadas:

- *Escherichia coli* productor de BLEE
- *Staphylococcus aureus:* R fosfomicina, R rifampicina, R de alto nivel mupirocina, R trimetoprim/sulfametaxol, S fusídico.
- *Enterococcus faecium:* R vancomicina, R teicoplanina, R de alto nivel estreptomicina, R de alto nivel gentamicina, R clindamicina, R ampicilina.
- *Acinetobacter baumannii*: R ciprofloxacina, R gentamicina, R ceftazidima, R cefotaxima, R meropenem, R tobramicina, R piperacilina.

Resultados del ensayo (tablas I, II, III).

Conclusión

El producto **F66 SR** (lote 2107/L1/2008) posee actividad bactericida, mostrando una reducción de la viabilidad superior a 10^5 a las concentraciones 40% y 80% en 5 minutos de contacto y a la concentración 80% en 1 minuto de contacto, para las cepas bacterianas y condiciones especificadas en el ensayo.

Madrid, a 29 de Octubre de 2008.

Dra. Raquel Andrade Lobato
Laboratorio de Higiene Hospitalaria
Servicio Medicina Preventiva

Dra. Beatriz Peláez Ros
Laboratorio de Higiene Hospitalaria
Servicio Medicina Preventiva

Dr. J. Fereres Castiel
Jefe de Servicio
Servicio Medicina Preventiva

Tabla I. Resultados del ensayo de validación en condiciones limpias, a la dilución más concentrada del ensayo (80%) y según las especificaciones indicadas en la norma.

Organismos del Ensayo	Ensayo de Validación			
	Suspensión Bacteriana	**Condiciones Experimentales**	**Procedimiento de Filtración**	**Método de Filtración**
Acinetobacter baumannii	V_c: 137;167 N_v: 1,5 x 10^3	V_c: 141; 160 A: 1,5 x 10^2	V_c: 130; 135 B: 1,3 x 10^2	V_c: 104; 125 C: 1.2 x 10^2
Escherichia coli	V_c; 270; 320 N_v: 3 x10^3	V_c: 290; 256 A: 2,7 x 10^2	V_c: 285; 277 B: 2,8x 10^2	V_c: 262; 281 C: 2,7 x 10^2
Staphylococcus aureus	V_c: 240;260 N_v: 2,5 x 10^3	V_c: 238; 242 A: 2,4 x 10^2	V_c: 199; 175 B: 1,9 x 10^2	V_c: 204; 206 C: 2 x 10^2
Enterococcus faecium	V_c; 240; 280 N_v: 2,6 x 10^3	V_c: 147; 135 A: 1,4 x 10^2	V_c: 140; 120 B: 1,3 x 10^2	V_c: 152; 146 C: 1,5 x 10^2

V_c= recuento viable
N_v= número de ufc/ml de la suspensión bacteriana. **N = 6,0 x 10^2 – 3,0 x 10^3 ufc/ml.**
A= número de ufc/ml obtenido en el ensayo de validación de las condiciones experimentales. **A≥0.05 N_v**
B= número de ufc/ml obtenido en el ensayo de validación del procedimiento de filtración. **B≥0.05 N_v**
C= número de ufc/ml obtenido en el ensayo de validación del método de filtración. **C≥0.5 B**

Tabla II. Resultados del test de ensayo en condiciones limpias, según las especificaciones indicadas en la norma (1 minuto de contacto).

Organismos del Ensayo	Suspensión Bacteriana de Ensayo	Procedimiento de Ensayo a la concentración % (V/V)			
			20%	**40%**	**80%**
Acinetobacter baumannii	10^{-5}: >300;>300 10^{-6}: 42; 49 N: 4,6 x 10^8	**V_c** **N_a** **R**	>300;>300 >3x10^3 <10^5	105;92 1x10^3 4.6x10^4	0;2 <1,5x10^2 >10^5
Escherichia coli	10^{-5}: >300;>300 10^{-6}: 46; 40 N: 4,3 x 10^8	**V_c** **N_a** **R**	>300;>300 >3x10^3 <10^5	>300;>300 >3x10^3 <10^5	1;0 <1,5x10^2 >10^5
Staphylococcus aureus	10^{-5}: >300;>300 10^{-6}: 44; 46 N: 4,5 x 10^8	**V_c** **N_a** **R**	>300;>300 >3x10^3 <10^5	>300;>300 >3x10^3 <10^5	0;2 <1,5x10^2 >10^5
Enterococcus faecium	10^{-5}: 179;127 10^{-6}: 24; 28 N: 2.6 x 10^8	**V_c** **N_a** **R**	>300;>300 >3x10^3 <10^5	21;14 3,5x10^2 7.4x10^4	0;0 <1,5x10^2 >10^5

N= número de ufc/ml de la suspensión bacteriana de ensayo. **N= 1,5 x 10^8 – 5,0 x 10^8 ufc/ml.**
V_c= recuento viable
N_a= número de ufc/ml en la mezcla de ensayo
R= reducción de la viabilidad

Tabla III. Resultados de los test de ensayo en condiciones limpias, según las especificaciones indicadas en la norma (5 minutos de contacto).

Organismos del Ensayo	Suspensión Bacteriana de Ensayo	Procedimiento de Ensayo a la concentración % (V/V)			
			20%	40%	80%
Acinetobacter baumannii	10^{-5}: >300;>300 10^{-6}: 42; 49 N: $4,6 \times 10^8$	**V_c** **N_a** **R**	0;0 <$1,5x10^2$ >10^5	0;1 <$1,5x10^2$ >10^5	0;0 <$1,5x10^2$ >10^5
Escherichia coli	10^{-5}: >300;>300 10^{-6}: 46; 40 N: $4,3 \times 10^8$	**V_c** **N_a** **R**	>300;>300 >$3x10^3$ <10^5	1;0 <$1,5x10^2$ >10^5	0;0 <$1,5x10^2$ >10^5
Staphylococcus aureus	10^{-5}: >300;>300 10^{-6}: 44; 46 N: $4,5 \times 10^8$	**V_c** **N_a** **R**	30;30 $3x10^2$ $1.5x10^5$	0;1 <$1,5x10^2$ >10^5	0;0 <$1,5x10^2$ >10^5
Enterococcus faecium	10^{-5}: 179;127 10^{-6}: 24; 28 N: 2.6×10^8	**V_c** **N_a** **R**	200;201 $2x10^3$ $1.3x10^4$	1;0 <$1,5x10^2$ >10^5	0;0 <$1,5x10^2$ >10^5

N= número de ufc/ml de la suspensión bacteriana de ensayo. **N= $1,5 \times 10^8 - 5,0 \times 10^8$ ufc/ml.**
V_c= recuento viable
N_a= número de ufc/ml en la mezcla de ensayo
R= reducción de la viabilidad

Universitat Autònoma de Barcelona

Departament de Ciència Animal i dels Aliments

RESULTADOS DE ANÁLISIS

Informe de la Norma UNE-EN-1276

Determinación de la actividad bactericida del producto en condiciones de utilización generales (condiciones sucias)

a) Identificación del laboratorio de ensayo: José Collado.

b) Identificación de la muestra:

- Nombre del Producto ..... F66SR.
- Número de lote..... 0210/L1/2008
- Condiciones de almacenamiento.....Refrigeración y oscuridad.
- Sustancia(s) activa(s) y su(s) concentraciones.....Peróxido de hidrógeno.

c) Método de ensayo y su validación:

- Método.....Dilución-neutralización.
- Neutralizante..... 6 g/l lecitina; 60 g/l polisorbato 80; 10 g/l tiosulfato sódico; 2 g/l L-histidina, 30 g/l saponina en disolución tampón fosfato de 0.25 mol/l al 1%. Solución esterilizada en autoclave.

d) Condiciones experimentales:

- Diluyente del producto utilizado en ensayo.....agua dura estéril (300 mg/kg CaCO3).
- Concentraciones de producto ensayadas.....80, 40 y 20% (V/V).
- Apariencia de los productos diluidos.....Soluble en agua dura.
- Sustancia de interferencia.....Solución acuosa de albúmina bovina: 3 g/l.
- Temperatura de ensayo .....20ºC ± 1ºC.
- Tiempo de contacto.....5 min ± 10 s.
- Temperatura de incubación.....37ºC ± 1ºC.
- Identificación de las cepas bacterianas utilizadas.....*Shigella sonnei*, *Salmonella typhimurium* ATCC 13311 y *Listeria monocytogenes* Scott A.

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josejuan.rodriguez@uab.cat
www.uab.es

e) Resultados del ensayo: ver Tabla 1.

f) Conclusión:

De conformidad con la norma UNE-EN-1276, el lote 0210/L1/2008 del producto F66SR, posee actividad bactericida después de cinco minutos a 20ºC en condiciones sucias (solución acuosa de albúmina bovina 3 g/l) para las cepas especificadas a continuación: *Shigella sonnei*, *Salmonella typhimurium* ATCC 13311 y *Listeria monocytogenes* Scott A.

Universitat Autónoma de Barcelona

Departament de Ciència Animal i dels Aliments

Dr. José Juan Rodríguez Jerez
Profesor Titular

Bellaterra (Cerdanyola del Vallès), catorce de noviembre de dos mil cinco

Tabla1. Resultados del Ensayo del Producto F66SR

Organismo de ensayo	Ensayo de validación				Suspensión bacteriana de ensayo	Procedimiento de ensayo a la concentración %(V/V)			
	Suspensión bacteriana (Validación)	Condiciones Experimen-tales	Control De toxicidad	Control del método de dilución-neutralización			20	40	80
Shigella sonnei	Vc: 71; 90 Nv: $1.2x10^3$	Vc: 78; 68 A: $7.7x10^2$	Vc: 65; 85 B: $7.5x10^2$	Vc: 65; 61 C: $6.0x10^2$	10^{-6}: 120; 140 N: $1,3x10^8$	V_c: N_a: R	10; 7 8.5×10^2 $> 10^5$	0; 1 $< 1.5 \times 10^2$ $> 10^5$	0; 0 $< 1.5x10^2$ $> 10^5$
Salmonella typhimurium ATCC 13311	Vc: 97; 110 Nv: $1.0x10^3$	Vc: 98; 98 A: $1,1x10^3$	Vc: 90; 102 B: $1.1x10^3$	Vc: 81; 96 C: $8.2x10^2$	10^{-6}: 140; 183 N: $1.6x10^8$	V_c: N_a: R	20; 18 1.9×10^2 $> 10^5$	0; 0 $< 1.5x10^2$ $> 10^5$	0; 0 $< 1.5x10^2$ $> 10^5$
Listeria monocytogenes Scott A	Vc: 85; 78 Nv: $9.5x10^2$	Vc: 40; 50 A: $5,5x10^2$	Vc: 60;50 B: $5,5x10^2$	Vc:40; 50 C: $4.1x10^2$	10^{-6}:180; 170 N: $1.8x10^8$	V_c: N_a: R	90; 80 $8.5x10^3$ $< 10^5$	0; 0 $< 1.5x10^2$ $> 10^5$	0; 0 $< 1.5x10^2$ $> 10^5$

V_c= recuento viable.
N= Número de ufc/ml de la suspensión bacteriana de ensayo.
N_V= número de ufc/ml de la suspensión bacteriana.
R= reducción de viabilidad.
N_a= número de ufc/ml en el ensayo de validación de las condiciones experimentales.
A= número de ufc/ml obtenido en el ensayo de validación de las condiciones experimentales.
B= número de ufc/ml obtenido en el ensayo de validación de la no toxicidad del neutralizador.
C= número de ufc/ml en el ensayo de validación de dilución-neutralización.

RCC CIDA Study Number S11338

F66 SR:

Acute Oral Toxicity Study in Rats
(Fixed Dose Method)

Report

Author: Mª Carmen López

Sponsor: José Collado, S.A.
Costa Rica, 35, Local 1
08027 - Barcelona
Spain

RCC

TABLE OF CONTENTS

1 PREFACE

1.1 GENERAL

Title	F66 SR: Acute Oral Toxicity Study in Rats (Fixed Dose Method)
Sponsor	José Collado, S.A. Costa Rica, 35, Local 1 08027 - Barcelona Spain Tel.: +34 93 349 61 12 Fax: +34 93 351 46 40
Study Monitor	Mª José Collado tecnico@josecollado.com
Test Facility	RCC CIDA S.A. Centro Industrial Santiga c/Argenters, 6 08130-Santa Perpètua de Mogoda Barcelona Spain Tel.: +34 93 719 03 61 Fax: +34 93 729 97 31

1.2 RESPONSIBILITIES

Study Director	Mª Carmen López mc.lopez@cidasal.com
Deputy Study Director	Sílvia López si.lopez@cidasal.com
Head of QAU	Francisca Crespí f.crespi@cidasal.com

1.3 SCHEDULE

Experimental Starting Date	13 February 2008
Experimental Completion Date	5 March 2008
Delivery of Animals	6 February 2008 (one female, 300 mg/kg) 6 February 2008 (one female, 2000 mg/kg) 13 February 2008 (four females, 2000 mg/kg)
Acclimatization	6 February to 12 February 2008 (one female, 300 mg/kg) 6 February to 14 February 2008 (one female, 2000 mg/kg) 13 February to 19 February 2008 (four females, 2000 mg/kg)

Sighting Study Starting Date Treatment	 13 February 2008 (one female, 300 mg/kg) 15 February 2008 (one female, 2000 mg/kg)
Main Study Starting Date Treatment	 20 February 2008 (four females, 2000 mg/kg)
Study Completion Date	Date of issue of Final Report

1.4 ARCHIVING

RCC CIDA S.A. will retain the study plan, raw data and the final report of the present Study.

All the above-mentioned items will be kept in the RCC CIDA S.A. archives for a minimum of five years starting from the issue date of the final report.

After this period, these materials will be sent to the Sponsor who will be responsible for keeping them during the period established by current legislation.

At the Sponsor's request, RCC CIDA S.A. may continue to keep them in their archives. A new contract will be issued stating the extra cost for storage.

1.5 SIGNATURE PAGE

Study Director: Mª Carmen López

.....

date: 9 May 2008

Test Facility Management: Agustín Casadesús

.....

date: 9. May 2008

1.6 QUALITY ASSURANCE STATEMENT (GLP)

RCC CIDA STUDY NUMBER:	S11338
TEST ITEM:	F66 SR
STUDY DIRECTOR:	Mª Carmen López
TITLE:	Acute Oral Toxicity Study in Rats (Fixed Dose Method)

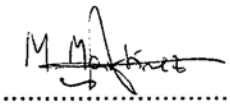
The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were periodically audited. The study plan and this report were audited by the Quality Assurance. The dates are given below.

Dates and Types of QA Inspections		Dates of Reports to the Study Director and Test Facility Management
21.Jan.08	Study Plan	21.Jan.08
20.Feb.08	Formulation, animal weighing, po administration and recording of clinical signs	20.Feb.08
05.Mar.08	Sacrifice and Necropsy	05.Mar.08
23.Apr.08	Final Report	23.Apr.08

This statement also confirms that this final report reflects the raw data.

Quality Assurance Auditor: Magda Martínez



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date: 9. May 2008

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE / GLP GUIDELINES

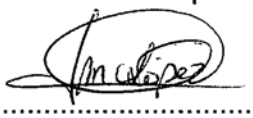
RCC CIDA STUDY NUMBER:	S11338
TEST ITEM:	F66 SR
STUDY DIRECTOR:	Mª Carmen López
TITLE:	Acute Oral Toxicity Study in Rats (Fixed Dose Method)

This study was carried out according to the principles of Good Laboratory Practice (GLP) specified in:

- Real Decreto (Royal Decree) 1369/2000 of 19 July (Spain)
- OECD Principles of Good Laboratory Practice (as revised in 1997), C (97) 186/Final, Paris, 26 November 1997
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004

There were no circumstances that may have affected the quality or integrity of the data.

Study Director: Mª Carmen López



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date: 9 May 2008

1.8 TEST GUIDELINES

The study procedures described in this report meet or exceed the requirements of the following guidelines:

- Commission Directive 2004/73/EC of 29 April 2004. Annex 2B. Method B.1 bis
- OECD Guideline for the Testing of Chemicals, Guideline 420 Acute Oral Toxicity. Fixed Dose Procedure, updated 17 December 2001.
- Commission Directive 2001/59/EC of 6 August 2001, Annex VI

1.9 ANIMAL WELFARE

The study procedures are in accordance with:

- Decret (Decree) 214/1997 of 30 July. Ministry of Agriculture, Livestock and Fishing of the Autonomous Government of Catalonia, Spain.
- Real Decreto (Royal Decree) 1201/2005 of 10 October (Spain).
- Council Directive 86/609/EEC of 24 November 1986.

The study procedures have been checked and approved by the Animal Experimentation Ethics Committee at RCC CIDA S.A.

The documentation generated was filed in the archives of the Animal Experimentation Ethics Committee at RCC CIDA S.A.

The least number of animals was used in compliance with current regulations and scientific integrity. The welfare of the animals was taken into account in terms of number and extent of procedures to be performed.

2 SUMMARY

In the main study, one group of five female Wistar Hannover HsdRccHan rats (including one used in the initial sighting study) was treated with F66 SR by oral gavage administration at a dosage of 2000 mg/kg body weight. The test item was diluted in the vehicle (distilled water) at a concentration of 0.2 g/mL and administered at a dosing volume of 10 mL/kg.

During the sighting study, one additional animal was dosed at 300 mg/kg without showing any signs of toxicity.

The animals were examined daily during the acclimatization period and mortality, viability and clinical signs were recorded. All animals were examined for clinical signs at approximately 30 minutes, 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2-15. Mortality/viability was recorded at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15. Body weights were recorded on day 1 (prior to administration) and on days 8 and 15. All animals were necropsied and examined macroscopically.

All animals survived until the end of the study period.

No clinical signs were observed during the course of the study.

The body weight of the animals was within the range commonly recorded for this strain and age.

No macroscopic findings were recorded at necropsy.

3 CONCLUSION

Based on the results obtained in the Study, and in accordance with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), the test item is classified in Category 5/Unclassified.

In accordance with the system currently used in the European Union (Directive 2004/73/EC, Annex 2B) and with the criteria published in the Official Journal of the European Communities of 6 August 2001 (Directive 2001/59/EC, Annex VI), it can be concluded that the test item F66 SR is considered UNCLASSIFIED and therefore it is not necessary to assign it a risk phrase.

4 PURPOSE

The purpose of this study was to assess the acute toxicity of F66 SR when administered by a single oral gavage to rats, followed by an observation period of 14 days.

This study provides information for both hazard assessment and hazard classification purposes.

5 MATERIALS AND METHODS

5.1 TEST SYSTEM

Test system	Wistar Hannover HsdRccHan: WIST rats
Rationale	Recognized by the international guidelines as a recommended test system.
Source	Harlan Italy Zona Industriale Azzima, 57 33049 San Pietro al Natisone Italy
Number of animals	6 females (nulliparous and non-pregnant)
Age when treated	Approximately 8–10 weeks
Body weight when treated	136–155 g
Identification	By means of an ear-punch technique
Acclimatization	From 7 to 9 days.
Veterinary inspection	During acclimatization, the animals were examined by a veterinary surgeon. Only animals without any visible signs of illness were used for the treatment.

5.2 HUSBANDRY

Room no.	117 and 115
Conditions	**Standard Laboratory Conditions.** Air-conditioned with target ranges for room temperature 20-24 °C, relative humidity 30-60% and 10-20 air changes per hour. Room temperature and relative humidity will be monitored continuously. Values outside these ranges occasionally occurred. These transient variations are considered not to have any influence on the study and therefore these data are not reported but are retained at RCC CIDA S.A. There was a 12-hour fluorescent light/12-hour dark cycle.
Accommodation	**Sighting study:** Groups of four at the most during acclimatization and individually during the study in Makrolon

	type-5 cages (59.0 x 38.5 x 20.0 cm) with Lignocel 3-4 sawdust bedding. **Main study:** Groups of three at the most during the acclimatization and groups of four during the study in Makrolon type-5 cages (59.0 x 38.5 x 20.0 cm) with Lignocel 3-4 sawdust bedding.
Diet	Pelleted standard HARLAN TEKLAD 2014C rat/mouse maintenance diet ad libitum (supplied by Harlan Interfauna Ibérica, S.L., batch no. 090607MA, expiry date: 4 March 2008). Results of analyses for contaminants are archived at RCC CIDA S.A.
Water	Tap water in bottles ad libitum. Results of bacteriological, chemical and contaminant analyses are archived at RCC CIDA S.A.

5.3 TEST ITEM

The following information was provided by the Sponsor:

Identification	F66 SR
Batch number	1112/L2/2007
Composition	7.99% active principle
Description	Disinfectant
Physical form/color	Blue liquid
Arrival date	14 December 2007
Expiry date	11 December 2008
Storage conditions	Refrigerator (2 to 8 °C)
Safety precautions	Routine hygienic procedures: gloves, goggles and facemask.

The remainder of the test item will be disposed of once the studies where the test item is used are over.

5.4 VEHICLE

Distilled water (Aqua B. Braun Ref. 387875, batch no.: 7364B05)

5.5 TEST ITEM PREPARATION

The necessary amount of test item was weighed in a volumetric flask and distilled water was added gradually until the desired volum is reached. The formulation was then transferred to a beaker and kept under agitation if necessary.

No correction factor for purity was applied.

5.6 TREATMENT

The animals received a single dose of the test item by oral gavage after being fasted for 11-12 hours, but with free access to water. Food was presented approximately between the interval comprised from 3 hours and a half to 4 hours after dosing.

The application volume was 10 mL/kg body weight.

Sighting study: Initially, one rat was treated at the dose of 300 mg/kg. Because no signs of toxicity were recorded in this animal, another animal was treated at the dose of 2000 mg/kg (see Appendix B).

Main study: Based on the information obtained in the Sighting Study, i.e. no evident toxicity at 300 mg/kg and no death at 2000 mg/kg, the test item was administered orally at the dose of 2000 mg/kg to four more animals, resulting in a group of five animals treated at this dose in the Main Study (see Appendix B).

Rationale	Oral administration was chosen because this is one possible route of human exposure during manufacture, handling and use of the test item.

5.7 OBSERVATIONS

Mortality / Viability	Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (in common with the clinical signs) and twice daily during days 2-15.
Body weights	On test days 1 (prior to administration), 8 and 15.
Clinical signs	Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Once daily during days 2-15.

6 NECROPSY

All the animals were killed at the end of the observation period by an intraperitoneal injection of sodium pentobarbital at the minimum dose of 200 mg/kg and volume of 10 mL/kg and discarded after macroscopic examinations were performed. No organs or tissues were retained.

7 STATISTICAL ANALYSIS

No statistical analysis was used.

8 DATA COMPILATION

All data were recorded on data sheets.

9 RESULTS

9.1 SIGHTING STUDY

One animal treated at 300 mg/kg did not show any signs of toxicity, i.e. no clinical signs or macroscopic findings were observed and body weight development was normal for this strain and age.

No macroscopic findings were recorded at necropsy.

The animal treated at 2000 mg/kg is reported under "9.2 MAIN STUDY".

9.2 MAIN STUDY

9.2.1 MORTALITY

No deaths occurred at the dosage level of 2000 mg/kg during the study.

9.2.2 CLINICAL SIGNS

No clinical signs were observed during the course of the study.

9.2.3 BODY WEIGHTS

The body weight of the animals was within the range commonly recorded for this strain and age.

9.2.4 MACROSCOPIC FINDINGS

No macroscopic findings were recorded at necropsy.

10 EVALUATION AND INTERPRETATION

Based on the results obtained in the Study, the test item has been classified according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), (see Appendix B).

It is also classified according to the system currently used in the European Union (see Appendix B).

The Risk Phrase is determined based on the classification currently used in the European Union, as follows:

Classification	Risk Phrase
VERY TOXIC	R 28
TOXIC	R 25
HARMFUL	R 22

APPENDIX A

INDIVIDUAL FINDINGS – MAIN STUDY

TABLE 1 Body weight (g): Main Study

Dose level (mg/kg)	Animal number	Sex	Day of the observation period			Weight at necropsy
			1	8	15	
2000	2	F	139	161	179	179
	3	F	136	165	177	177
	4	F	154	185	197	197
	5	F	139	164	183	183
	6	F	155	186	202	202
		Mean	144.6	172.2	187.6	187.6
		SD	9.13	12.24	11.22	11.22

TABLE 2 Macroscopic findings: Main Study

Dose level (mg/kg)	Animal number	Sex	Mode of Death	Findings
2000	2	F	S	No abnormal findings
	3	F	S	No abnormal findings
	4	F	S	No abnormal findings
	5	F	S	No abnormal findings
	6	F	S	No abnormal findings

S: scheduled necropsy, D: found dead, K: killed *in extremis*

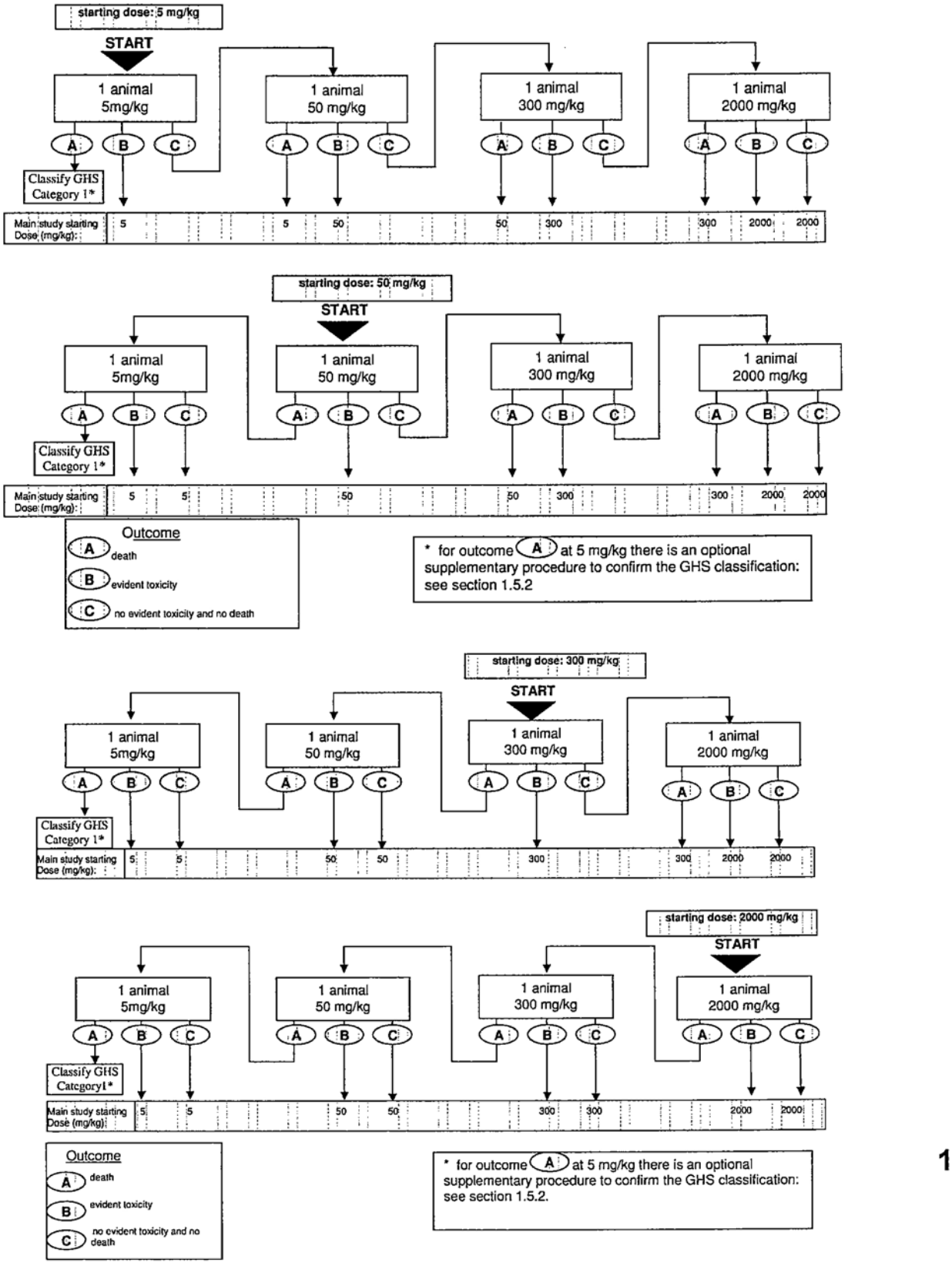
TABLE 3: Individual findings (clinical observations expressed as the number of affected animals): Main Study
Dose level: 2000 mg/kg

OBSERVATION PERIOD	Day of treatment					Days following treatment													
(Days)	hours					2	3	4	5	6	7	8	9	10	11	12	13	14	15
Post-administration time	0-0.5	1	2	3	5														
No. of surviving animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
No anomaly detected	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Irritability																			
Lethargy																			
Unconsciousness																			
Increased motor activity																			
Decreased motor activity																			
Abnormal gait																			
Decreased muscle tone																			
Prostration																			
Hunched back																			
Opisthotonos																			
Tremors																			
Clonic convulsions																			
Tonic convulsions																			
Apnea																			
Brachypnea																			
Tachypnea																			
Dyspnea																			
Cyanosis																			
Pallor																			
Rubescence																			
Piloerection																			
Alopecia																			
Salivation																			
Pigmented orbital secretion																			
Pigmented snout																			
Diarrhea																			
Palpebral ptosis																			

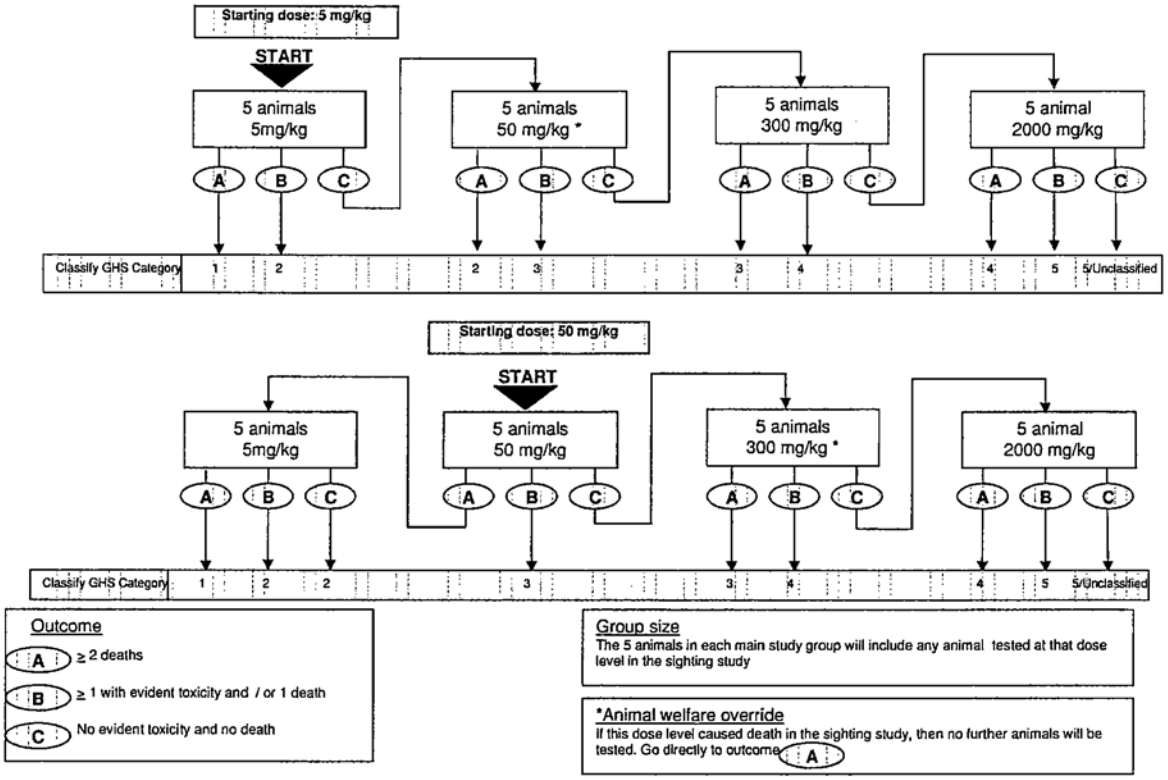
APPENDIX B

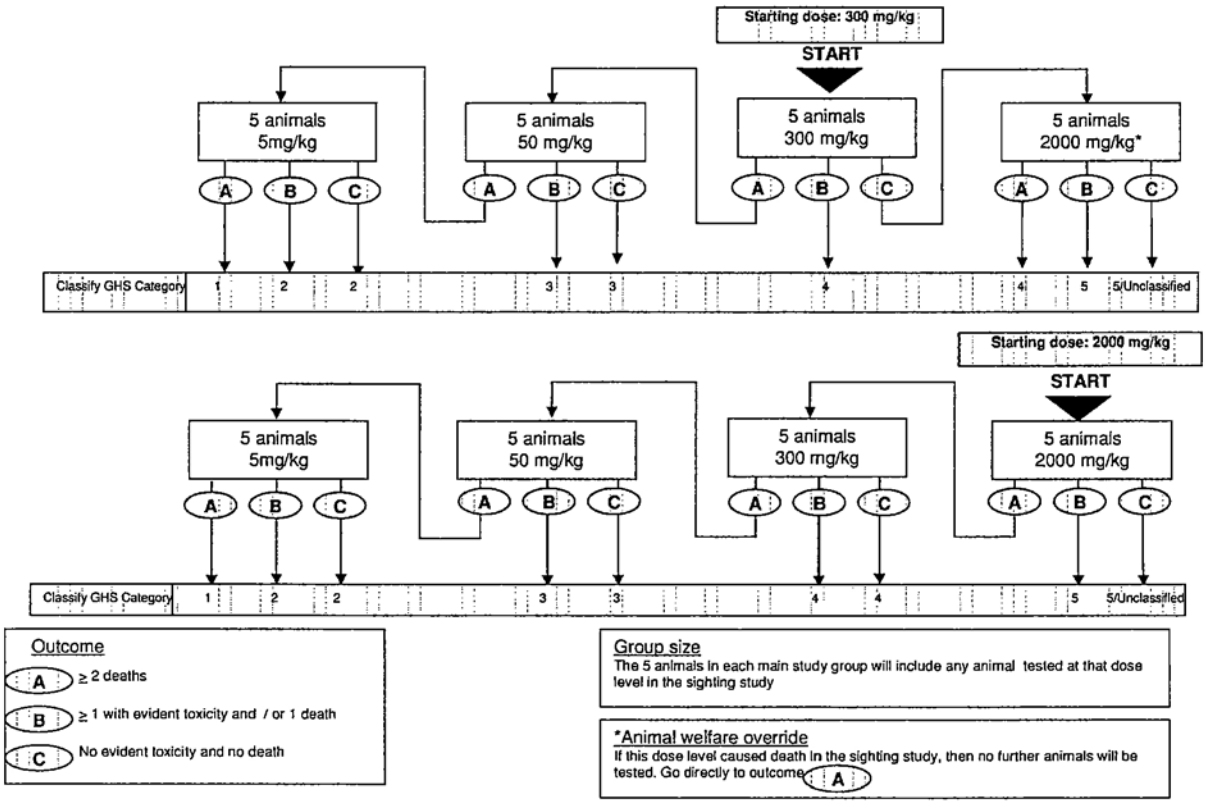
INTERPRETATION OF RESULTS

FLOW CHART FOR THE SIGHTING STUDY

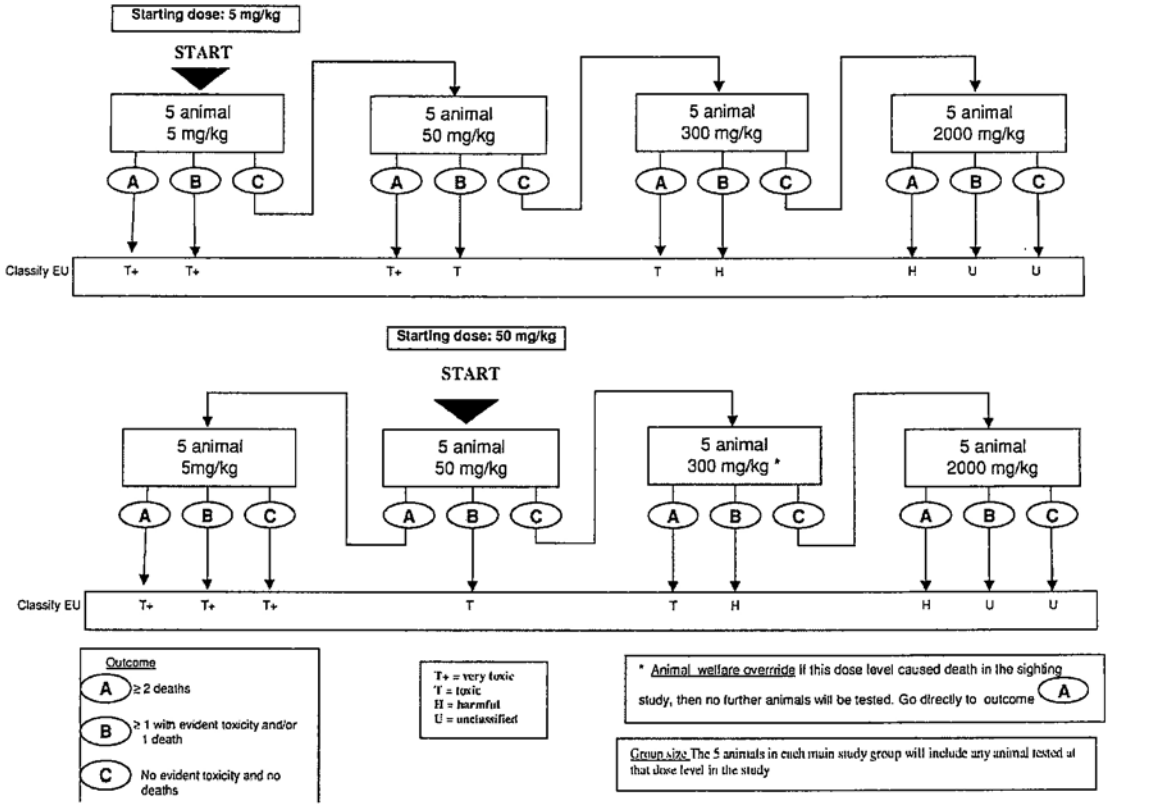


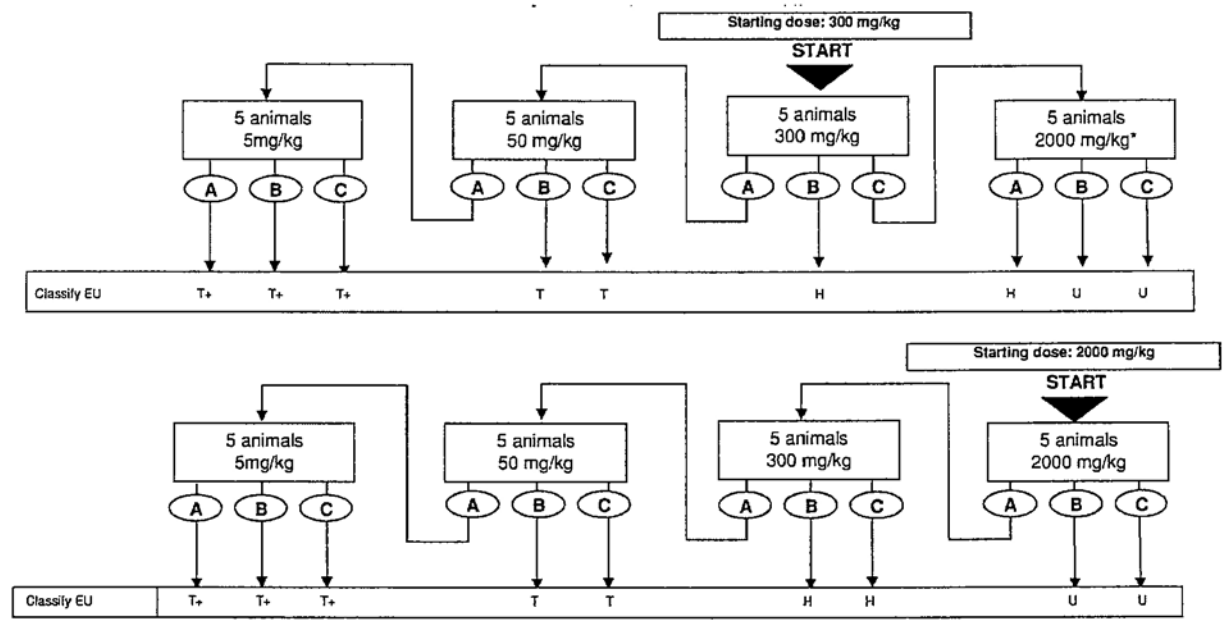
FLOW CHART FOR THE MAIN STUDY





GUIDANCE ON CLASSIFICATION ACCORDING TO THE EU SCHEME TO COVER THE TRANSITION PERIOD UNTIL FULL IMPLEMENTATION OF THE GLOBALLY HARMONISED CLASSIFICATION SYSTEM (GHS)





RCC CIDA Study Number S11362

F66 SR:

Primary Skin Irritation Study in Rabbits
(4-Hour Semi-Occlusive Application)

Report

Author: Mª Carmen López

Sponsor: José Collado, S.A.
Costa Rica, 35, Local 1
08027-Barcelona
Spain

RCC

TABLE OF CONTENTS

1 PREFACE

1.1 GENERAL

Title	F66 SR: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)
Sponsor	José Collado, S.A. Costa Rica, 35, Local 1 08027-Barcelona Tel.: +34 93 349 61 12 Fax:: +34 93 351 46 40
Study Monitor	Mª José Collado tecnico@josecollado.com
Test Facility	RCC CIDA S.A. Centro Industrial Santiga C/Argenters, 6 08130-Santa Perpètua de Mogoda Barcelona Spain Tel.: +34 93 719 03 61 Fax: +34 93 729 97 31

1.2 RESPONSIBILITIES

Study Director	Mª Carmen López mc.lopez@cidasal.com
Deputy Study Director	Sílvia López si.lopez@cidasal.com
Head of QAU	Francisca Crespí f.crespi@cidasal.com

1.3 SCHEDULE

Experimental Starting Date	12 February 2008
Experimental Completion Date	29 February 2008
Acclimatization	25 January to 11 February 2008 (one male) 25 January to 18 February 2008 (two males)
Treatment	12 February 2008 (one male) 19 February 2008 (two males)
Observation of local findings	Throughout 10 days after treatment
Study Completion Date	Date of issue of Final Report

1.4 ARCHIVING

RCC CIDA S.A. will retain the study plan, raw data and the final report of the present Study.

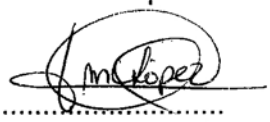
All the above-mentioned items will be kept in the RCC CIDA S.A. archives for a minimum of five years starting from the issue date of the final report.

After this period, these materials will be sent to the Sponsor who will be responsible for keeping them during the period established by current legislation.

At the Sponsor's request, RCC CIDA S.A. may continue to keep them in their archives. A new contract will be issued stating the extra cost for storage.

1.5 SIGNATURES

Study Director: Mª Carmen López



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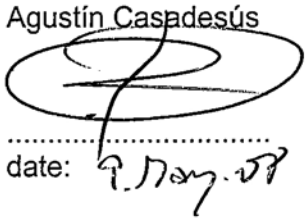
date: 9 May 2008

Test Facility Management: Agustín Caspadesús



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date: 9 May 08

1.6 QUALITY ASSURANCE STATEMENT (GLP)

RCC CIDA STUDY NUMBER: S11362

TEST ITEM: F66 SR

STUDY DIRECTOR: Mª Carmen López

TITLE: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures, were periodically audited. The study plan and this report were audited by the Quality Assurance. The dates are given below.

Dates and Types of QA Inspections		Dates of Reports to the Study Director and Test Facility Management
21.Jan.08	Study plan	21.Jan.08
05.Feb.08	Formulation, administration and dermal evaluation*	05.Feb.08
28.Apr.08	Final report	28.Apr.08

* Process-based inspection

This statement also confirms that this final report reflects the raw data.

Quality Assurance Auditor: Núria Jornet

Núria

.....

date: 9 May 08

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE/GLP GUIDELINES

RCC CIDA STUDY NUMBER:	S11362
TEST ITEM:	F66 SR
STUDY DIRECTOR:	Mª Carmen López
TITLE:	Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)

This study was carried out according to the principles of Good Laboratory Practice (GLP) specified in:

- Real Decreto (Royal Decree) 1369/2000 of 19 July (Spain)
- OECD Principles of Good Laboratory Practice (as revised in 1997), C (97) 186/Final, Paris, 26 November 1997
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004

There were no circumstances that may have affected the quality or integrity of the data.

Study Director: Mª Carmen López

.....

date: 9 May 2008

1.8 TEST GUIDELINES

The study procedures described in this report meet or exceed the requirements of the following guidelines:

- OECD Guideline for the Testing of Chemicals, Guideline 404 Acute Dermal Irritation/Corrosion, 24 April 2002.
- Commission Directive 2004/73/EC of 29 April 2004. Annex 2D. Method B.4. Acute Toxicity: Dermal Irritation/Corrosion.
- Commission Directive 2001/59/EC of 6 August 2001, Annex VI. General Classification and Labelling Requirements for Dangerous Substances and Preparations.

1.9 ANIMAL WELFARE

The study procedures are in accordance with:

- Decret (Decree) 214/1997 of 30 July. Ministry of Agriculture, Livestock and Fishing of the Autonomous Government of Catalonia, Spain.
- Real Decreto (Royal Decree) 1201/2005 of 10 October (Spain).
- Council Directive 86/609/EEC of 24 November 1986.

The study procedures have been checked and approved by the Animal Experimentation Ethics Committee at RCC CIDA S.A.

The documentation generated was filed in the archives of the Animal Experimentation Ethics Committee at RCC CIDA S.A.

The least number of animals was used in compliance with current regulations and scientific integrity. The welfare of the animals was taken into account in terms of number and extent of procedures to be performed.

1.10 CLASSIFICATION GUIDELINES

Commission Directive 2001/59/EC adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, August 06, 2001 (Official Journal of the European Communities Nr. L 225/1, August 21, 2001).

2 SUMMARY

The primary skin irritation potential of F66 SR was investigated according to OECD test guideline no. 404. The test item was applied by topical semi-occlusive application of 0.5 mL to the intact flank of each of three young adult New Zealand White rabbits. The duration of treatment was four hours. The scoring of skin reactions was performed 1, 24, 48 and 72 hours, as well as 7 and 10 days after removal of the dressing.

The mean score was calculated across three scoring times (24, 48 and 72 hours after patch removal) for each animal for erythema/eschar grades and for edema grades, separately. The mean erythema/eschar scores of the three animals were 1.00, 1.33 and 2.00 and the mean edema score was 0.00 for all the animals.

The mean values at 24, 48 and 72 hours following administration for each type of lesion for all three animals together were:

Erythema: 1.44

Edema: 0.00

The application of F66 SR to the skin resulted in signs of mild irritation. These included well-defined erythema and very slight edema (in all animals) at 1 hour after removing the patch. The erythema remitted to very slight erythema (grade 1) in all the animals on day 7 of observation and disappeared completely in all the animals 10 days after removing the patch. The very slight edema disappeared in all animals at 24 hours after removing the patch. Slight dryness of the skin and slight desquamation were recorded in one animal at 72 hours and 7 days, respectively. These effects were reversible and were no longer evident 10 days after treatment, the end of the observation period for all animals. The test item caused no staining of the treated skin. No corrosive effects were noted on the treated skin of any animal at any of the measuring intervals and no clinical signs were observed.

Thus, the test item did not induce significant or irreversible damage to the skin.

Based upon the referred classification criteria (Commission Directive 2001/59/EC of August 2001), F66 SR is considered to be "NOT IRRITATING" to rabbit skin and, therefore, does not require any risk phrase.

3 PURPOSE

The purpose of this primary skin irritation study was to assess the possible irritation potential when a single dose of F66 SR was placed on the skin of rabbits for approximately four hours.

This study should provide a rational basis for risk assessment in humans, as skin contact is one of the possible routes of human exposure.

The test item was administered at 0.5 mL/patch, the dose specified in the test guidelines for a liquid test item.

4 MATERIALS AND METHODS

4.1 TEST SYSTEM

Test system	Young Adult New Zealand White Rabbit
Rationale	Recognized by the international guidelines as the recommended test system.
Source	Granja San Bernardo, S.L. Ctra. de Tarazona, s/n 31522-Tulebras Navarra (Spain)
Total number of animals	3
Sex	Males
Age at start of treatment	13-14 weeks
Body weight at start of treatment	2.9-3.0 kg
Identification	By numbered ear tags
Acclimatization	From 18 to 25 days
Veterinary inspection	During acclimatization, the animals were examined by a veterinary surgeon. Only animals without any visible signs of illness were used for the treatment.

4.2 HUSBANDRY

Room number	Room 107
Conditions	**Standard Laboratory Conditions** Air-conditioned with ranges for room temperature of 22-25 °C and for relative humidity of 35-55% and 10-20 air changes per hour. Room temperature and relative humidity were monitored continuously. Values outside these ranges occasionally occurred. These transient variations are considered not to have any influence on the study and, therefore, these data are not reported but are retained at RCC CIDA S.A. There was a 12-hour fluorescent light/12-hour dark cycle.
Accommodation	Individual in stainless steel cages (52 x 58 x 43 cm). The cages were identified by a card stating animal number, sex and date of arrival, study number, Study Director's name, test item identification, dose level, administration route, and date of treatment.
Diet	Pelleted standard Teklad 2030 rabbit diet ad libitum (supplied by Harlan Interfauna Ibérica, S.L., Ctra. Sant Miquel del Fai, km 3, Apartado 38, 08182-Sant Feliu de Codines, Barcelona (Spain); batches 081507M, 209911 and 110807MA, expiry dates, 11 February, March and 6 May 2008, respectively). Results of analyses for contaminants are archived at RCC CIDA S.A.
Water	Tap water in bottles ad libitum. Results of bacteriological, chemical and contaminant analyses are archived at RCC CIDA S.A.

4.3 TEST ITEM

The following information was provided by the Sponsor:

Identification	F66 SR
Batch number	1112/L2/2007
Composition	7.99% active principle
Description	Disinfectant
Physical form/color	Blue liquid
Arrival date	14 December 2007
Expiry date	11 December 2008
Storage conditions	Refrigerator (2 to 8 °C)

Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

The remainder of the test item will be disposed of once the studies are completed and the final reports have been issued.

4.4 TEST ITEM PREPARATION

0.5 mL (per application) of F66 SR was measured with a syringe and applied undiluted as received from the Sponsor.

The pH of the test item was measured and was found to be 3.19.

According to Commission Directive 2004/73/EC, B.4. and OECD Guideline 404, a test item needs not to be tested if the pH value is less than 2 or greater than 11.5, owing to its predictable corrosive properties.

4.5 TREATMENT

The skin of the animals was examined one day before treatment and was clipped with an electric clipper. The skin of the animals used in the study was in good condition.

On the day of treatment, 0.5 mL of F66 SR was placed on a surgical gauze patch (2.5 cm x 2.5 cm). This gauze patch was applied to the intact skin of the clipped area. The patch was covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and anchored with tape.

The duration of treatment was 4 hours after which the dressing was removed and the skin flushed with distilled water.

As it was suspected that the test item might produce severe irritancy/corrosion, a single animal was treated first. Three patches were applied sequentially. The first patch was removed at 3 minutes. In the absence of severe cutaneous reaction, a second patch was applied to another test area and was removed after 1 hour. No severe cutaneous reaction was observed, therefore, a third patch was applied to another test area and was removed after 4 hours. As neither a corrosive effect nor a severe irritant effect was observed after a four-hour exposure, the test was completed using the two remaining animals, each with one patch only, for an exposure period of four hours. After each removal of dressing, the treated skin areas were flushed with distilled water.

4.6 OBSERVATIONS

Viability/Mortality	Daily from acclimatization of the animals to termination of the test.
Clinical signs	Daily during acclimatization, on the day of application and at each observation time point.
Body weights	On the day of application and at each observation time point.
Skin reaction	See Sections 4.7 and 4.8.

4.7 IRRITATION SCORES

The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, April 29 2004 (see section 9.3).

4.8 SKIN OBSERVATION TIME POINTS AND TERMINATION

The skin reaction was assessed at approximately 1, 24, 48 and 72 hours, as well as 7, and 10 days after exposure (removal of the dressing, gauze patch and test item).

4.9 TREATMENT OF RESULTS

Data were summarized in tabular form, showing for each animal the irritation scores for erythema and edema at all measurement intervals. Any lesions were described, including the degree and nature of irritation, corrosion or any other toxic effects observed, and their reversibility.

The mean score was calculated across three scoring times (24, 48 and 72 hours after patch removal) for each animal for erythema/eschar grades and for edema grades, separately.

The test item was graded according to the scoring system published in the Official Journal of the European Communities on 6 August 2001 (Directive 2001/59/EC, Annex VI).

A substance or a preparation is considered irritant and is assigned the risk phrase R38 if the inflammation of the skin lasts for at least 24 hours and shows some of the following values:

- Mean value for the whole of the group:
  - Erythematous lesions: ≥2
  - Edematous lesions: ≥2
- or damage equivalent to the mean values stated above, calculated for each animal, observed in at least two animals.

Corresponding mean values are calculated based on all the mean values obtained at each observation time (24, 48 and 72 hours) for each type of lesion.

Skin inflammation is also considered important if it persists in at least two animals at the end of the observation period. Special effects such as hyperplasia, desquamation, decoloration, formation of fissures, scabs or alopecia, should also be taken into account.

A substance or a preparation is considered corrosive if it produces tissular damage in all the skin layers of at least one animal.

It is considered to produce burns and is assigned the risk phrase R34 if tissular lesions appear throughout all the skin layers after an exposure time of not more than four hours.

If tissular lesions appear throughout all the skin layers in an exposure time of not more than three minutes the test item is considered to produce serious burns and is assigned the risk phrase R35.

5 NECROPSY

The animals were killed by intravenous injection of sodium pentobarbital into the ear vein at a dose of at least 60 mg/kg body weight and at volume of 1 mL/kg and then discarded.

No necropsy was performed.

6 DATA COMPILATION AND STATISTICAL ANALYSIS

All data was recorded on data sheets.

No statistical analysis was performed.

7 RESULTS

7.1 VIABILITY/MORTALITY/CLINICAL SIGNS

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred.

7.2 IRRITATION

The mean score was calculated across three scoring times (24, 48 and 72 hours after patch removal) for each animal for erythema/eschar grades and for edema grades, separately. The mean erythema/eschar scores for the three animals were 1.00, 1.33 and 2.00 and the mean edema score was 0.00 for all three.

The mean values at 24, 48 and 72 hours following administration for each type of lesion for all three animals together were:

Erythema: 1.44

Edema: 0.00

At 1 hour after removing the patch, well-defined erythema (grade 2) and very slight edema (grade 1) were recorded in all animals. The well-defined erythema persisted in one animal until 72 hours after treatment, becoming very slight (grade 1) on day 7 after treatment. In the other two animals, the well-defined erythema became very slight (grade 1) at 24 hours until 7 days after administration. However one of them still showed well-defined erythema (grade 2) at the 48-hour observation. The very slight edema disappeared completely in all animals at 24 hours after administration.

Slight dryness of the skin and slight desquamation were recorded in one animal at 72 hours and 7 days after treatment, respectively.

No abnormal findings were observed on the treated skin of any animal 10 days after treatment, the end of the observation time.

7.3 COLORATION

No staining produced by the test item of the treated skin was observed.

7.4 CORROSION

No alterations of the treated skin were observed nor were corrosive effects evident on the skin.

7.5 BODY WEIGHTS

The body weights of all rabbits were considered to be within the normal range of variability.

Body weights (kg)		Day of treatment	Recording time after treatment				
			hours			days	
Animal no.	Sex		24	48	72	7	10
3208	male	2.88	2.85	2.92	2.97	3.14	3.27
3203	male	2.96	3.00	3.04	3.10	3.22	3.25
3204	male	2.92	3.07	3.07	3.18	3.34	3.38

8 CONCLUSION

Based upon the cited classification criteria (Commission Directive 2001/59/EC of August 2001), F66 SR is considered to be "NOT IRRITATING" to rabbit skin and, therefore, does not require any risk phrase.

9 APPENDICES

9.1 SKIN IRRITATION SCORES

Key to Symbols:

M Male

Note: Commission Directive 2004/73/EC, April 29, 2004, Grading of Skin Reactions is presented on page 21.

TABLE 1: SKIN IRRITATION SCORES - INDIVIDUAL VALUES

Animal Number	Sex	Evaluation Interval*	Erythema	Edema
3208	M		2	1
3203	M	1 hour	2	1
3204	M		2	1
3208	M		1	0
3203	M	24 hours	1	0
3204	M		2	0
3208	M		1	0
3203	M	48 hours	2	0
3204	M		2	0
3208	M		1	0
3203	M	72 hours	1	0
3204	M		2	0
3208	M		1	0
3203	M	7 days	1	0
3204	M		1	0
3208	M		0	0
3203	M	10 days	0	0
3204	M		0	0

* Examinations were performed at the specified times after removal of the dressing.

TABLE 2: SKIN IRRITATION SCORES – MEAN SCORES AFTER 24, 48 AND 72 HOURS

Animal Number	Sex	Erythema	N	Edema	N
3208	M	1.00	3	0.00	3
3203	M	1.33	3	0.00	3
3204	M	2.00	3	0.00	3
Mean score		1.44		0.00	

N=number of available data points.

TABLE 3: SKIN IRRITATION SCORES – ASSESSMENT ACCORDING TO EEC GUIDELINES

Evaluated intervals	Erythema	Edema
24 hours 48 hours 72 hours	Not Irritating	Not Irritating

9.2 INDIVIDUAL FINDINGS

ANIMAL NO. 3208, MALE

After 1 hour:	Erythema:	Well-defined
	Edema:	Very slight
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 24 hours:	Erythema:	Very slight
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 48 hours:	Erythema:	Very slight
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 72 hours:	Erythema:	Very slight
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 7 days:	Erythema:	Very slight
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 10 days:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED

INDIVIDUAL FINDINGS

ANIMAL NO. 3203, MALE

After 1 hour:	Erythema:	Well-defined
	Edema:	Very slight
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 24 hours:	Erythema:	Very slight
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 48 hours:	Erythema:	Well defined
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 72 hours:	Erythema:	Very slight
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 7 days:	Erythema:	Very slight
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 10 days:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED

INDIVIDUAL FINDINGS

ANIMAL NO. 3204, MALE

After 1 hour:	Erythema:	Well-defined
	Edema:	Very slight
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 24 hours:	Erythema:	Well-defined
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 48 hours:	Erythema:	Well-defined
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 72 hours:	Erythema:	Well-defined
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED
After 7 days:	Erythema:	Very slight
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	Slight
	Staining:	NO ABNORMAL FINDINGS NOTED
After 10 days:	Erythema:	NO ABNORMAL FINDINGS NOTED
	Edema:	NO ABNORMAL FINDINGS NOTED
	Desquamation:	NO ABNORMAL FINDINGS NOTED
	Staining:	NO ABNORMAL FINDINGS NOTED

9.3 SUMMARY OF EVALUATION CRITERIA

COMMISSION DIRECTIVE 2004/73/EC, APRIL 29, 2004

Grading of Skin Reactions

ERYTHEMA AND ESCHAR FORMATION

No erythema 0

Very slight erythema (barely perceptible) 1

Well-defined erythema 2

Moderate to severe erythema 3

Severe erythema (beef redness) to eschar formation preventing grading of erythema 4

EDEMA FORMATION

No edema 0

Very slight edema (barely perceptible) 1

Slight edema (edges of area well-defined by definite raising) 2

Moderate edema (edges raised approximately 1 mm) 3

Severe edema (raised more than 1 mm and extending beyond the area of exposure) 4

RCC CIDA Study Number S11395

F66 SR:

Primary Eye Irritation Study in Rabbits

Report

Author: Mª Carmen López

Sponsor: José Collado, S.A.
Costa Rica, 35, Local 1
08027 - Barcelona
Spain

TABLE OF CONTENTS

1 PREFACE

1.1 GENERAL

Title	F66 SR: Primary Eye Irritation Study in Rabbits
Sponsor	José Collado, S.A. Costa Rica, 35, Local 1 08027-Barcelona Spain Tel.: +34 93 349 61 12 Fax: +34 93 351 46 40
Study Monitor	Mª José Collado tecnico@josecollado.com
Test Facility	RCC CIDA S.A. Centro Industrial Santiga c/Argenters, 6 08130-Santa Perpètua de Mogoda Barcelona Spain Tel.: +34 93 719 03 61 Fax: +34 93 729 97 31

1.2 RESPONSIBILITIES

Study Director	Mª Carmen López mc.lopez@cidasal.com
Deputy Study Director	Sílvia López si.lopez@cidasal.com
Head of QAU	Francisca Crespí f.crespi@cidasal.com

1.3 SCHEDULE

Experimental Starting Date	18 February 2008
Acclimatization	8 February to 17 February 2008 (one male) 8 February to 2 March 2008 (one male) 8 February to 10 March 2008 (one male)
Treatment	18 February 2008 (one male) 3 March 2008 (one male) 11 March 2008 (one male)
Observation of local findings	Throughout 7 days after treatment (one male). Throughout 17 days after treatment (one male). Throughout 21 days after treatment (one male).

Termination	10 March 2008 (two males) 28 March 2008 (one male)
Study Completion Date	Date of issue of Final Report

1.4 ARCHIVING

RCC CIDA S.A. will retain the study plan, raw data and the final report of the present Study.

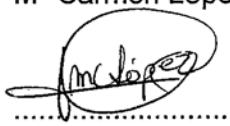
All the above-mentioned items will be kept in the RCC CIDA S.A. archives for a minimum of five years starting from the issue date of the final report.

After this period, these materials will be sent to the Sponsor who will be responsible for keeping them during the period established by current legislation.

At the Sponsor's request, RCC CIDA S.A. may continue to keep them in their archives. A new contract will be issued stating the extra cost for storage.

1.5 SIGNATURE PAGE

Study Director: Mª Carmen López



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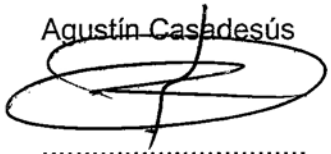
date: 9 June 2008

Test Facility Management: Agustín Casadesús



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date: 9 June 2008

1.6 QUALITY ASSURANCE STATEMENT (GLP)

RCC CIDA STUDY NUMBER: S11395

TEST ITEM: F66 SR

STUDY DIRECTOR: Mª Carmen López

TITLE: Primary Eye Irritation Study in Rabbits

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

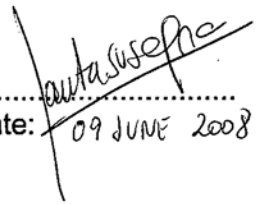
Study procedures were periodically audited. The study plan and this report were audited by the Quality Assurance. The dates are given below.

Dates and Types of QA Inspections		Dates of Reports to the Study Director and Test Facility Management
21.Jan.08	Study plan	21.Jan.08
11.Mar.08	Formulation (ocular)*	11.Mar.08
11.Mar.08	Ocular administration*	11.Mar.08
11.Mar.08	Ocular evaluation*	11.Mar.08
14.May.08	Final report	14.May.08

* Process-based inspection

This statement also confirms that this final report reflects the raw data.

Quality Assurance Auditor: Carme Santasusagna



date: 09 JUNE 2008

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE/GLP GUIDELINES

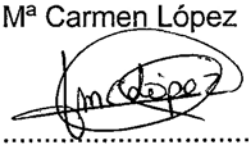
RCC CIDA STUDY NUMBER:	S11395
TEST ITEM:	F66 SR
STUDY DIRECTOR:	Mª Carmen López
TITLE:	Primary Eye Irritation Study in Rabbits

This study was carried out according to the principles of Good Laboratory Practice (GLP) specified in:

- Real Decreto (Royal Decree) 1369/2000 of 19 July (Spain)
- OECD Principles of Good Laboratory Practice (as revised in 1997), C (97) 186/Final, Paris, 26 November 1997
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004

There were no circumstances that may have affected the quality or integrity of the data.

Study Director: Mª Carmen López



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date: 9 June 2008

1.8 TEST GUIDELINES

The study procedures described in this report meet or exceed the requirements of the following guidelines:

- OECD Guideline for the Testing of Chemicals, Guideline 405 Acute Eye Irritation/Corrosion, 24 April 2002
- Commission Directive 2004/73/EC of 29 April 2004. Annex 2E. Method B.5. Acute Toxicity: Eye Irritation/Corrosion
- Commission Directive 2001/59/EC of 6 August 2001, Annex VI. General Classification and Labelling Requirements for Dangerous Substances and Preparations.

1.9 ANIMAL WELFARE

The study procedures are in accordance with:

- Decret (Decree) 214/1997 of 30 July. Ministry of Agriculture, Livestock and Fishing of the Autonomous Government of Catalonia, Spain.
- Real Decreto (Royal Decree) 1201/2005 of 10 October (Spain).
- Council Directive 86/609/EEC of 24 November 1986.

The study procedures have been checked and approved by the Animal Experimentation Ethics Committee at RCC CIDA S.A.

The documentation generated was filed in the archives of the Animal Experimentation Ethics Committee at RCC CIDA S.A.

The least number of animals was used in compliance with current regulations and scientific integrity. The welfare of the animals was taken into account in terms of number and extent of procedures to be performed.

1.10 CLASSIFICATION GUIDELINES

Commission Directive 2001/59/EC adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, August 06, 2001 (Official Journal of the European Communities Nr. L 225/1, August 21, 2001).

2 SUMMARY

The primary eye irritation potential of F66 SR was investigated according to OECD test guideline no. 405. The test item was applied by instillation of 0.1 mL into the right eye of each of three young adult New Zealand White rabbits. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours; 7 days (all the animals); 10, 14 and 17 days (two animals) and 21 days (one animal) after test item instillation.

The mean score was calculated across three scoring times (24, 48 and 72 hours after instillation) for each animal for corneal opacity, iris, redness and chemosis of the conjunctivae, separately. The individual mean scores for corneal opacity were 2.67, 0.00 and 0.67 and for iris were 1.00, 0.00 and 0.00. The individual mean scores for the conjunctivae were 3.00, 1.33 and 3.00 for reddening and 3.00, 0.67 and 1.67 for chemosis.

The mean values at 24, 48 and 72 hours following administration for each type of lesion for all three animals together were:

Corneal opacity:	1.11
Lesions in the iris:	0.33
Reddening:	2.44
Chemosis:	1.78

The instillation of F66 SR into the eye resulted in severe early-onset and partially reversible ocular changes such as opacity, lesions in the iris (in one animal), reddening and chemosis of the conjunctivae and discharge. These effects were not totally reversible after 21 days in one of the animals and were no longer evident on days 7 and 17 in the other two animals, respectively, which was the end of the observation period for each animal.

One hour after application, moderate redness (grade 2) was recorded in two animals and marked redness (grade 3) in the remaining one. Likewise, obvious swelling (grade 2) and ocular discharge, from slight to moderate, were observed in all three animals.

One animal showed moderate opacity (grade 3) at the 24- and 48-hour readings and slight opacity (grade 2) at the 72-hour reading. Only one of the other two animals showed very slight opacity (grade 1) at the 24- and 48-hour observations. Twenty-one days after instillation, very slight opacity (grade 1) was still present in one animal.

Delayed/reduced light reflex was observed at the 72-hour reading in one animal. At 24 and 48 hours, the iris was not assessable due to corneal opacity in the same animal.

Two animals showed marked or beefy redness (grade 3) at the 24-, 48- and 72-hour readings and the other animal showed moderate redness (grade 2) at the 24-hour observation as well as slight conjunctival redness (grade 1). At the 24-hour reading, marked swelling (with half-closed to closed lids, grade 4) was observed in one animal, marked swelling (with half-closed lids, grade 3) was recorded in another animal and obvious swelling with partial eversion of lids (grade 2) was noted in the third animal.

These lesions decreased progressively in all the animals.

Slight discharge was observed in one animal at the 24-hour reading and moderate discharge in two animals at the 24-hour observation and only in one of them at the 48- and 72-hour readings. In one of these animals, slight discharge was also recorded at 48 and 72 hours. Whitish secretion was recorded in two animals at 24 and 72 hours and in one of them at the 48-hour reading as well.

No staining of the treated eyes by the test item and no clinical signs were observed.

Thus, the test item induced significant and irreversible damage to the rabbit eye.

Based upon the classification criteria referred to previously (Commission Directive 2001/59/EC of August 06, 2001) and although the mean value of the corneal opacity was <3 and of the lesion in iris was <1.5, F66 SR is considered to "cause severe ocular lesions" to the rabbit eye, given that some lesions persisted 21 days after application and, therefore, requires the risk phrase R41.

3 PURPOSE

The purpose of this primary eye irritation study was to assess the possible irritation potential when a single dose of F66 SR was placed in the conjunctival sac of rabbit eyes.

This study should provide a rational basis for risk assessment in humans, as ocular contact is one of the possible routes of human exposure.

The test item was applied at 0.1 mL/animal, the dose specified in the test guidelines for a liquid test item.

4 MATERIALS AND METHODS

4.1 TEST SYSTEM

Test system	Young Adult New Zealand White Rabbit
Rationale	Recognized by the international guidelines as the recommended test system.
Source	Granja San Bernardo, S.L. Ctra. de Tarazona, s/n 31522-Tulebras Navarra (Spain)
Total number of animals	3
Sex	Males
Age at start of treatment	11-16 weeks
Body weight at start of treatment	2.7-3.0 kg
Identification	By numbered tags in the ears.
Acclimatization	From 10 to 32 days.
Veterinary inspection	During acclimatization, the animals were examined by a veterinary surgeon. Only animals without any visible signs of illness were used for the treatment.

4.2 HUSBANDRY

Room number	Room 107 and 110
Conditions	**Standard Laboratory Conditions** Air-conditioned with ranges for room temperature of 20 - 26 °C and for relative humidity of 30-55% and 10-20 air changes per hour. Room temperature and relative humidity were monitored continuously. Values outside these ranges occasionally occurred. These transient variations are considered not to have any influence on the study and therefore these data are not reported but are retained at RCC CIDA S.A. There was a 12-hour fluorescent light/12-hour dark cycle.
Accommodation	Individually in stainless steel cages (52 x 58 x 43 cm and 45 x 59 x 37 cm). The cages were identified by a card stating animal number, sex and date of arrival, study number, Study Director's name, test item identification, dose level, administration route, and date of treatment.
Diet	Pelleted standard Teklad 2030 Global Rabbit diet (supplied by Harlan Interfauna Ibérica, S.L., Ctra. Sant Miquel del Fai, km 3, Apartado 38, 08182-Sant Feliu de Codines, Barcelona, Spain; Batches: 081507 M, 210266 and 201501; expiry dates: 11 February 2008, June 2008 and September 2008) was offered ad libitum. Results of analyses for contaminants and composition are archived at RCC CIDA S.A.
Water	Tap water ad libitum by means of an automatic drinking system. Results of bacteriological, chemical and contaminant analyses were archived at RCC CIDA S.A.

4.3 TEST ITEM

The following information was provided by the Sponsor:

Identification	F66 SR
Batch number	1112/L2/2007
Composition	7.99% active principle
Description	Disinfectant
Physical form/color	Blue liquid
Arrival date	14 December 2007
Expiry date	11 December 2008
Storage conditions	Refrigerator (2 to 8 °C)
Safety precautions	Routine hygienic procedures: gloves, goggles and facemask

The remainder of the test item will be disposed of when the studies in which it is used are completed unless otherwise requested by the Sponsor.

4.4 TEST ITEM PREPARATION

0.1 mL (per animal) of F66 SR was measured with a syringe and applied undiluted as received from the Sponsor.

The pH of the test item was measured for a previous study (RCC CIDA Study number S11362: *Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)* with F66 SR) and was found to be 3.19.

According to Commission Directive 2004/73/EC, B.5. and OECD Guidelines 405, a test item does not need to be tested if the pH is less than 2 or greater than 11.5, owing to its predictable corrosive properties.

4.5 TREATMENT

The animals' eyes were examined before test item administration. The eyes of all animals used in the study were in good condition.

On the day of treatment, 0.1 mL of F66 SR was placed in the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of test item. The left eye remained untreated and served as the reference control.

Immediately after the administration, a plastic collar was placed on the rabbits for 1 hour to prevent them from rubbing their eyes.

As an irritant effect was observed in the first animal, a second animal was administered instead of exposing two additional animals simultaneously. As neither a corrosive effect nor a severe irritant effect was observed in the second animal, the test was completed using the remaining animal.

4.6 OBSERVATIONS

Viability/Mortality	Daily from acclimatization to the termination of test.
Body weights	On the day of application and in each observation time point.
Clinical signs	Daily during acclimatization, on the day of application and at each observation time point.
Eye reaction	See Sections 4.7 and 4.8.

4.7 IRRITATION SCORES

The eye reactions were assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, April 29, 2004 (see Appendix). Ocular discharge and the area of cornea involved were also assessed.

Individual findings recorded for each animal may differ slightly from the exact descriptions listed in the Directive.

4.8 EYE OBSERVATION TIME POINTS AND TERMINATION

The eyes of each animal were examined approximately 1, 24, 48, 72 hours; 7 days (all the animals); 10, 14 and 17 days (two animals) and 21 days (one animal) after test item instillation.

Except at the reading at 1 hour after administration, the corneal observation was always completed following instillation of an aqueous solution of 2% sodium fluorescein (®Colircusí Fluoresceína, Alcon Cusí, S.A., Batches No.: 7AJA1B and 7AJA1C, expiry date: March 2010) and subsequent washing with 0.9% physiological saline. Once the excess fluorescein had been removed, the corneal alterations were observed with the aid of a transilluminator with a cobalt blue filter.

The treated eyes were not rinsed after instillation of the test item.

4.9 TREATMENT OF RESULTS

Data were summarized in tabular form, showing the irritation scores for each animal at the designated observation time, a description of the degree and nature of irritation and the presence of serious lesions. The scores of each animal at the following reading times (24, 48, 72 hours) were used to calculate the respective mean values for each type of lesion.

The classification system was based upon the classification criteria referred to previously Commission Directive 2001/59/EC of August 06, 2001 (see Appendix).

5 NECROPSY

The animals were sacrificed at termination of observation by intravenous injection of sodium pentobarbital into the ear vein at a minimum dose of 60 mg/kg body weight and a volume of 1 mL/kg and discarded.

No necropsies were performed.

6 DATA COMPILATION AND STATISTICAL ANALYSIS

All data was recorded on data sheets.

No statistical analysis was performed.

7 RESULTS

7.1 VIABILITY/MORTALITY AND CLINICAL SIGNS

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred.

7.2 IRRITATION

The mean score was calculated across three scoring times (24, 48 and 72 hours after instillation) for each animal for corneal opacity, iris, redness and chemosis of the conjunctivae, separately. The individual mean scores for corneal opacity were 2.67, 0.00 and 0.67, and for iris were 1.00, 0.00 and 0.00. The individual mean scores for the conjunctivae were 3.00, 1.33 and 3.00 for reddening and 3.00, 0.67 and 1.67 for chemosis.

The mean values at 24, 48 and 72 hours following administration for each type of lesion for all three animals together were:

Corneal opacity:	1.11
Lesions in the iris:	0.33
Reddening:	2.44
Chemosis:	1.78

One hour after instillation, moderate redness of the conjunctivae (grade 2) was recorded in two animals and marked or beefy redness (grade 3) in the other. Obvious swelling with partial eversion of lids (grade 2) was observed in all the animals. One animal showed moderate discharge (grade 2) and the other two slight discharge (grade 1).

Moderate opacity (grade 3) was recorded in one animal at the 24- and 48-hour observations persisting as slight opacity (grade 2) at the 72-hour reading and affecting greater than three quarters up to whole corneal area in all cases. Another animal showed very slight opacity (grade 1) affecting one quarter (or less) but not zero at the 24- and 48-hour observations. Iris was not assessable due to corneal opacity in one animal at the 24- and 48-hour readings and this animal showed delayed/reduced light reflex at the 72-hour observation time. At the 24-, 48- and 72-hour readings, marked or beef redness (grade 3) was recorded in two animals. Moderate conjunctival redness (grade 2) 24 hours after instillation and slight redness (grade 1) 48 and 72 hours after instillation were recorded in the other animal. Marked swelling (with half-closed to closed lids, grade 4) at the 24-hour reading decreasing to marked swelling (with half-closed lids, grade 3) at 48 hours and obvious swelling with partial eversion of lids (grade 2) at 72 hours were recorded in one animal. Another animal showed marked swelling (with half-closed lids, grade 3) at the 24-hour reading decreasing to obvious swelling with partial eversion of lids (grade 2) 48 hours after treatment. The remaining animal showed obvious swelling with partial eversion of lids (grade 2) only at the 24-hour observation. Slight discharge was observed in one animal at the 24-hour reading and moderate discharge in two other animals at the 24-hour observation and in one of them also at the 48- and 72-hour readings. In one of these animals, slight discharge was also recorded at 48 and 72 hours. Whitish secretion was recorded in two animals at 24 and 72 hours and in one of them at the 48-hour reading as well.

The slight opacity (grade 2) persisted in one animal on day 7 and very slight opacity (grade 1) affecting a corneal area of one quarter (or less) but not zero was recorded in the observations between the 10-day and 21-day recordings. On days 7 and 10, one animal showed slight redness (grade 1) and another showed moderate conjunctival redness (grade 2) on day 7 decreasing to slight redness (grade 1) at the 10- and 14-day recordings. Slight swelling of the conjunctivae (grade 1) was noted in the 7-day observation in one animal.

These effects were irreversible in one of the animals (after 21 days) but were no longer evident on days 7 and day 17, respectively, for the other two animals. This was the end of the observation period for each animal.

7.3 COLORATION

No staining of the treated eyes produced by the test item was observed.

7.4 CORROSION

Corneal corrosion was observed, as one of the animals showed lesions in the cornea which were not totally reversible at 21 days after treatment.

7.5 BODY WEIGHTS

The body weights of all rabbits were considered to be within the normal range of variability.

Body weights in kg		Day of Treatment	Recording time after Treatment							
Animal number	Sex		24 hours	48 hours	72 hours	7 days	10 days	14 days	17 days	21 days
3517	male	2.70	2.73	2.80	2.85	3.07	3.15	3.22	3.33	3.48
3503	male	2.83	2.83	2.86	2.90	3.06				
3509	male	2.97	2.95	2.98	2.99	3.22	3.36	3.42	3.50	

8 CONCLUSION

Based upon the classification criteria referred to previously (Commission Directive 2001/59/EC of August 06, 2001) and although the mean value of the corneal opacity was <3 and of the lesion in iris was <1.5, F66 SR is considered to "cause severe ocular lesions" to the rabbit eye, given that some lesions persisted 21 days after application and, therefore, requires the risk phrase R41.

9 APPENDICES

9.1 EYE IRRITATION SCORES

Key to Symbols:

M Male

Note: Commission Directive 2004/73/EC, April 29, 2004, Grading of Ocular Lesions is presented on page 26 and used for classification under the Commission Directive 2001/59/EC, August 06, 2001 on page 28.

TABLE 1: EYE IRRITATION SCORES - INDIVIDUAL VALUES

Animal Number	Sex	Evaluation Interval*	Corneal Opacity	Area of Corneal Opacity	Iris	Conjunctivae	
						Redness	Chemosis
3517	M	1 hour	0	-	0	2	2
3503	M		0	-	0	2	2
3509	M		0	-	0	3	2
3517	M	24 hours	3	4	n.a.	3	4
3503	M		0	0	0	2	2
3509	M		1	1	0	3	3
3517	M	48 hours	3	4	n.a.	3	3
3503	M		0	0	0	1	0
3509	M		1	1	0	3	2
3517	M	72 hours	2	4	1	3	2
3503	M		0	0	0	1	0
3509	M		0	0	0	3	0
3517	M	7 days	2	1	0	1	1
3503	M		0	0	0	0	0
3509	M		0	0	0	2	0
3517	M	10 days[1]	1	1	0	1	0
3503	M		-	-	-	-	-
3509	M		0	0	0	1	0
3517	M	14 days	1	1	0	0	0
3503	M		-	-	-	-	-
3509	M		0	0	0	1	0
3517	M	17 days	1	1	0	0	0
3503	M		-	-	-	-	-
3509	M		0	0	0	0	0
3517	M	21 days[2]	1	1	0	0	0
3503	M		-	-	-	-	-
3509	M		-	-	-	-	-

- Not assessable because no fluorescein was used

* Examinations were performed at the specified times after instillation/application of the test item.

n.a. = not assessable due to corneal opacity.

[1]: Animal no. 3503 was not examined 10 days after treatment as no signs of irritation were present at the 7-day reading.

[2]: Animal no. 3509 was not examined 21 days after treatment as no signs of irritation were present at the 17-day reading.

TABLE 2: EYE IRRITATION SCORES – MEAN VALUES AFTER 24, 48 AND 72 HOURS

Animal Number	Sex	Corneal Opacity	N	Iris	N	Conjunctivae Redness	N	Conjunctivae Chemosis	N
3517	M	2.67	3	1.00	1	3.00	3	3.00	3
3503	M	0.00	3	0.00	3	1.33	3	0.67	3
3509	M	0.67	3	0.00	3	3.00	3	1.67	3
Mean score		1.11		0.33		2.44		1.78	

N = number of available data points.

TABLE 3: EYE IRRITATION SCORES – ASSESSMENT ACCORDING TO EC GUIDELINES

Evaluated intervals	Corneal Opacity	Iris	Conjunctivae Redness	Conjunctivae Chemosis
24 hours 48 hours 72 hours	**Not Irritating**	**Not Irritating**	**IRRITATING**	**Not Irritating**

9.2 INDIVIDUAL FINDINGS

ANIMAL NO. 3517, MALE

After 1 hour:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	moderately reddened, obvious swelling with partial eversion of lids
	Discharge:	moderate
	Test item:	NO REMNANTS EVIDENT
After 24 hours:	Cornea:	moderate opacity, affecting an area greater than three quarters up to whole area
	Iris:	not assessable due to corneal opacity
	Conjunctivae:	markedly reddened, beefy red, marked swelling (with half-closed to closed lids)
	Discharge:	moderate
	Test item:	NO REMNANTS EVIDENT
After 48 hours:	Cornea:	moderate opacity, affecting an area greater than three quarters up to whole area
	Iris:	not assessable due to corneal opacity
	Conjunctivae:	markedly reddened, beefy red, marked swelling (with half-closed lids)
	Discharge:	slight
	Test item:	NO REMNANTS EVIDENT
After 72 hours:	Cornea:	slight opacity, affecting an area greater than three quarters up to whole area
	Iris:	delayed/reduced light reflex
	Conjunctivae:	markedly reddened; beefy red, obvious swelling with partial eversion of lids
	Discharge:	slight
	Test item:	NO REMNANTS EVIDENT
After 7 days:	Cornea:	slight opacity, affecting an area of one quarter (or less) but not zero
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	slightly reddened, slight swelling
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT

Observations included: cornea, conjunctivae (including nictitating membrane) and iris. The presence (or absence, as appropriate) of opacity, vascularization, reddening, edema, discharge, staining and test item remnants were assessed.

INDIVIDUAL FINDINGS

ANIMAL NO. 3517, MALE

After 10 days:	Cornea:	very slight opacity, affecting an area of one quarter (or less) but not zero
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	slightly reddened
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT
After 14 days:	Cornea:	very slight opacity, affecting an area of one quarter (or less) but not zero
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	NO ABNORMAL FINDINGS NOTED
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT
After 17 days:	Cornea:	very slight opacity, affecting an area of one quarter (or less) but not zero
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	NO ABNORMAL FINDINGS NOTED
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT
After 21 days:	Cornea:	very slight opacity, affecting an area of one quarter (or less) but not zero
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	NO ABNORMAL FINDINGS NOTED
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT

Observations included: cornea, conjunctivae (including nictitating membrane) and iris. The presence (or absence, as appropriate) of opacity, vascularization, reddening, edema, discharge, staining and test item remnants were assessed.

INDIVIDUAL FINDINGS

ANIMAL NO. 3503, MALE

After 1 hour:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	moderately reddened, obvious swelling with partial eversion of lids
	Discharge:	slight
	Test item:	NO REMNANTS EVIDENT
After 24 hours:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	moderately reddened, obvious swelling with partial eversion of lids
	Discharge:	slight
	Test item:	NO REMNANTS EVIDENT
After 48 hours:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	slightly reddened
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT
After 72 hours:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	slightly reddened
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT
After 7 days:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	NO ABNORMAL FINDINGS NOTED
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT

Observations included: cornea, conjunctivae (including nictitating membrane) and iris. The presence (or absence, as appropriate) of opacity, vascularization, reddening, edema, discharge, staining and test item remnants were assessed.

INDIVIDUAL FINDINGS

ANIMAL NO. 3509, MALE

After 1 hour:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	markedly reddened, beefy red, obvious swelling with partial eversion of lids
	Discharge:	slight
	Test item:	NO REMNANTS EVIDENT
After 24 hours:	Cornea:	very slight opacity, affecting an area of one quarter (or less) but not zero
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	markedly reddened, beefy red, marked swelling (with half-closed lids)
	Discharge:	moderate
	Test item:	NO REMNANTS EVIDENT
After 48 hours:	Cornea:	very slight opacity, affecting an area of one quarter (or less) but not zero
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	markedly reddened, beefy red, obvious swelling with partial eversion of lids
	Discharge:	moderate
	Test item:	NO REMNANTS EVIDENT
After 72 hours:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	markedly reddened, beefy red
	Discharge:	moderate
	Test item:	NO REMNANTS EVIDENT
After 7 days:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	moderately reddened
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT

Observations included: cornea, conjunctivae (including nictitating membrane) and iris. The presence (or absence, as appropriate) of opacity, vascularization, reddening, edema, discharge, staining and test item remnants were assessed.

INDIVIDUAL FINDINGS

ANIMAL NO. 3509, MALE

After 10 days:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	slightly reddened
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT
After 14 days:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	slightly reddened
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT
After 17 days:	Cornea:	NO ABNORMAL FINDINGS NOTED
	Iris:	NO ABNORMAL FINDINGS NOTED
	Conjunctivae:	NO ABNORMAL FINDINGS NOTED
	Discharge:	NO ABNORMAL FINDINGS NOTED
	Test item:	NO REMNANTS EVIDENT

Observations included: cornea, conjunctivae (including nictitating membrane) and iris. The presence (or absence, as appropriate) of opacity, vascularization, reddening, edema, discharge, staining and test item remnants were assessed.

9.3 SUMMARY OF EVALUATION CRITERIA

- Commission Directive 2004/73/EC, April 29, 2004
- Commission Directive 2001/59/EC, August 06, 2001

COMMISSION DIRECTIVE 2004/73/EC, APRIL 29, 2004

Grading of Ocular Lesions

CORNEA

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

No ulceration or opacity 0
Very slight*:
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible 1
Slight*: Easily discernible translucent area, details of iris slightly obscured 2
Moderate*: Nacreous area, no details of iris visible, size of pupil barely discernible 3
Severe*: Opaque cornea, iris not discernible through the opacity 4

IRIS

Normal 0
Delayed*/reduced light reflex*:
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive) 1
Absent light reflex*:
No reaction to light, hemorrhage, gross destruction (any or all of these) 2

CONJUNCTIVAE

Redness (refers to most severe reading of palpebral and bulbar conjunctivae when compared with control eye)

Blood vessels normal 0
Slightly*: Some blood vessels definitely hyperemic (injected) 1
Moderately*: Diffuse, crimson color, individual vessels not easily discernible 2
Markedly*: Diffuse beefy red 3

Chemosis: lids and/or nictitating membranes

No swelling 0
Slight*: Any swelling above normal (including nictitating membranes) 1
Obvious*: Obvious swelling with partial eversion of lids 2
Marked*: Swelling with lids about half-closed 3
Marked*: Swelling with lids more than half-closed 4

*:brief description of grading not indicated in Directive

ADDITIONALLY, OCULAR DISCHARGE AND AREA OF CORNEAL OPACITY WERE ASSESSED ACCORDING TO THE FOLLOWING SCHEME:

OCULAR DISCHARGE

No discharge 0
Slight: Any amount different to normal (does not include small amount observed in inner canthus of normal animal) 1
Moderate: Discharge with moistening of the lids and hair just adjacent to the lids 2
Marked: Discharge with moistening of the lids and hairs, and a considerable area around the eye (running) 3

CORNEAL OPACITY

Area of cornea involved
Zero 0
One quarter (or less) but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4

COMMISSION DIRECTIVE 2001/59/EC, AUGUST 06, 2001

The following risk phrases shall also be assigned in accordance with the criteria given:

"R36 - Irritating to eyes"

Substances and preparations which, when applied to the eye of the animal, cause significant ocular lesions which occurred within 72 hours after exposure and which persist for at least 24 hours.

Ocular lesions are significant if the mean scores of the eye irritation test cited in Annex V have any of the following values:

- cornea opacity equal to or greater than 2 but less than 3;
- iris lesion equal to or greater than 1 but not greater than 1,5;
- redness of the conjunctivae equal to or greater than 2,5;
- oedema of the conjunctivae (chemosis) equal to or greater than 2;

or, in the case where the Annex V test has been completed using three animals if the lesions, on two or more animals, are equivalent to any of the above values except that for iris lesion the value should be equal to or greater than 1 but less than 2 and for redness of the conjunctivae the value should be equal to or greater than 2,5.

In both cases all scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating the respective mean values.

Substances or preparations which cause significant ocular lesions, based on practical experience in humans.

Organic peroxides except where evidence to the contrary is available.

"R41 - Risk of serious damage to eyes"

Substances and preparations which, when applied to the eye of the animal, cause severe ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours.

Ocular lesions are severe if the mean scores of the eye irritation test in Annex V have any of the following values:

- cornea opacity equal to or greater than 3;
- iris lesion greater than 1,5.

The same shall be the case where the test has been completed using three animals if the lesion, on two or more animals, have any of the values:

- cornea opacity equal to or greater than 3;
- iris lesion equal to 2.

In both cases all scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating the respective mean values.

Ocular lesions are also severe when they are still present at the end of the observation time.

Ocular lesions are also severe if the substance or preparation causes irreversible coloration of the eyes.

Substances and preparations which cause severe ocular lesions, based on practical experience in humans.