#### The use of corneas from animals of different age in the Bovine Corneal Opacity and Permeability (BCOP) assay.

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#### BCOP results obtained with corneas from:

- 1) adult animals (> 24 months)
- 2) young animals (6 8 months)

#### Methodology

After background opacity measurement, medium was removed from the anterior compartment and corneas were treated with 0.75 ml of the test solution. Corneas (3 per group) were treated for 10 minutes followed by a 120 minutes recovery period. Medium was removed from the anterior compartment and replaced by 1 ml of a 0.4% sodium-fluorescein solution. Corneas were incubated in a horizontal position for 90 minutes at  $32^{\circ}C$  in a water-bath. After incubation, medium from the posterior chamber was removed and its optical density (OD) determined with a spectrophotometer at 490 nm. In Vitro Score = opacity + [15 x permeability]

#### Code of each compound is recorded on each raw data sheet

>>> compound **17** (acetone) need to be repeated since results did not comply with previously collected data in our laboratory. Due to the high vapor pressure of acetone (201.57 mmHg @ 22.0 °C), a technical artefact could have occurred...

Code	Compound	CAENO	In vivo	In vivo	In V	itro BCOF	) (>24 mo	nths)	In Vit	tro BCOP	COP (6 - 8 months)	
Code	compound	CAS NO.	EU	GHS	Opacity	Perm.	IVS	Class	Opacity	Perm.	IVS	Class
1	3,3-dimethylpentane	562-49-2	NI	NI	0.6	0.01	0.8	NON	0.0	0.02	0.3	NON
2	3-methoxy-1,2-propanediol	623-39-2	NI	NI	-0.3	0.00	0.2	NON	0.6	0.02	0.9	NON
3	polyethylene glycol 400	25322-68-3	NI	NI	-0.3	0.00	-0.3	NON	0.0	0.08	1.1	NON
4	glycerol	56-81-5	NI	NI	-1.0	0.01	-0.9	NON	-0.7	-0.01	-0.8	NON
5	methyl cyclopentane	96-37-7	NI	NI	1.0	0.43	7.5	MILD	1.3	0.26	5.2	MILD
6	tween 20	9005-64-5	NI	NI	0.0	0.01	0.1	NON	0.0	-0.01	-0.1	NON
7	methyl <i>iso</i> -butyl ketone	108-10-1	NI	NI	6.6	1.07	22.7	MILD	5.7	0.83	18.1	MILD
8	toluene	108-88-3	NI	NI	6.3	3.18	54	MOD	6.0	1.46	28.0	MOD
9	methyl amyl ketone	110-43-0	NI	NI	5.3	1.80	32.3	MOD	4.0	0.99	18.8	MILD
10	2-methyl-1-pentanol	105-30-6	NI	2B	12.0	4.30	76.6	SEV	8.6	1.94	37.7	MOD
11	ethanol	64-17-5	NI	2B	16.0	2.34	51	MOD	16.3	1.83	43.8	MOD
12	sodium hydroxide (1%)	1310-73-2	R36	2B	99.7	4.16	162	SEV	135.7	3.74	191.8	SEV
13	triton X-100 (5%)	9002-93-1	R36	2B	4.3	3.81	61.5	SEV	4.7	3.70	60.1	SEV
14	1-octanol	111-87-5	R36	2B	10.0	5.24	88.6	SEV	10.3	1.53	33.3	MOD
15	2-ethyl-1-hexanol	104-76-7	R36	2B	4.3	1.76	30.6	MOD	2.3	0.86	15.3	MILD
16	n-hexanol	111-27-3	R36	2A	15.3	3.73	71.2	SEV	14.0	3.62	68.2	SEV
17	acetone	67-64-1	R36	2A	39**	2.95	83.2	SEV	91.3	2.86	134.2	SEV
18	cyclohexanol	108-93-0	R41	1	15.3	5.04	90.7	SEV	11.6	2.13	43.6	MOD
19	cetylpyridinium bromide (6%)	140-72-7	R41	1	11.7	1.01	26.8	MOD	15.0	1.66	39.9	MOD
20	benzalkonium chloride (10%)	8001-54-5	R41	1	92.2	4.22	155.4	SEV	105.7	4.05	166.5	SEV

#### The use of corneas from animals of different age in the Bovine Corneal Opacity and Permeability (BCOP) assay.

#### **Prediction Model**

BCOP In Vitro Score	Class
<u>&lt;</u> 3	NON
3.1-25	MILD
25.1-55	MOD
> 55.1	SEV

#### \*\* to be repeated (technical artefact probably occurred)

Compounds 1 → 20
 Adult animals (>24 months)

#### Calculation of the in vitro eye irritation score for liquids

Test art	icle			3,3-Dime	ethylpenta	ne [562-4	9-2]					
<b>Batch</b> N	0.			14502CN								
Concent	ration			100%		Treatment time 10 min						
Code				A1	(1)							
Sequence In			Intern 8E				OP	OP-KIT				
No.	Treatment		Opacit	ty at			Permeability	7	In vitro score			
Cornea		t0	t120	t120 - t0								
1	NC	2	2	0	1		0.006		0.1			
2	MEM	1	1	0	1		0.012		0.2			
3	100%	1	3	2	1		0.009		2.1			
	Mean ± S.D.			0.7	± 1.2		$0.009 \pm 0.003$	1	$0.8 \pm 1.1$			
						8		-				
					Correcte	ed value	Correct	ed value				
4	Test article	1	3	2	1.3		0.023 0.014		1.5			
5		0	1	1	0.3		0.018 0.009		0.4			
6	100%	1	2	1	0.3		0.014 0.005	-	0.4			
<u> </u>	Mean ± S.D.				0.6	± 0.6	0.009	+ 0.005	$0.8 \pm 0.6$			
	Mean ± 5.D.				0.0	_ 0.0	0.002	_ 0.000	5.0 _ 5.0			

NC: Negative Control

REMARKS	Filter	OPACIT	Y
	1 A	75 B	-75
	2 A	154 B	-157
	3 A	250 B	-255

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Test art	icle			3-methox	(y-1,2-pro	spanediol 9	8%						
<b>Batch</b> N	lo.			05307-078	3								
Concen	tration			100%		Treatment time 2 hours							
Code				B1	2)								
Sequen	Sequence Intern				A	1			OP-K	(IT			
Intern			Intern 2 -		1			01 1					
					_								
No.	Treatment		Opacit	ty at			Perm	neability		In vitro score			
Cornea		tO	t120	t120 - t0									
					1								
1	NC	0	0	0	1		0.004	1	ſ	0.1			
2			0	0			0.004	1		0.1			
2		0	1	0			0.006	-	-	0.1			
3	100%	0	1	1		•	0.010			1.2			
	Mean $\pm$ S.D.			0.3	± 0.6		0.007	$\pm 0.003$		$0.5 \pm 0.6$			
					Correcte	ed value		Corrected value					
4	Test article	0	0	0	-0.3		0.019	0.012	I	-0.1			
5	1	0	0	0	-0.3	1	0.066	0.059	ľ	0.6			
6	100%	0	0	0	-0.3		0.032	0.025		0.0			
	Mean + S D				-0.3	+ 00	0.052	0.023 + 0.02		0.2 + 0.4			
	Mean 1 5.D.	1			-0.5	± 0.0		$0.032 \pm 0.02$	4	0.2 ± 0.4			

NC: Negative Control

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REMARKS	Filter OPACI	TY
	1 A B	
	2 A B	
	3 A B	

Paraph

Date 31-Jan-00

Test art	icle			Polyethylene glycol 400									
Batch N	0.			3H0110	<b>U</b>								
Concent	tration			100%	~	Treatme	ent time 10 mi	n					
Code				C1	(3)								
Sequence 11A					0			OP	-KIT				
					_								
No. Treatment Opac			Opacit	ty at			Permeability		In vitro score				
Cornea		t0	t120	t120 - t0									
					_								
1	NC	0	0	0			0.002		0.0				
2	NaC1 0.9%	0	0	0			0.003		0.0				
3	100%	1	1	0		_	0.001		0.0				
	Mean ± S.D.			0.0	± 0.0		$0.002 \pm 0.001$	L .	$0.0 \pm 0.0$				
			'			-							
					Correcte	ed value	Correc	ted value					
4	Test article	0	0	0	0.0		0.000 -0.00	2	0.0				
5		0	0	0	0.0		0.003 0.001	L	0.0				
6	100%	1	0	-1	-1.0		0.010 0.008	3	-0.9				
	Mean ± S.D.				-0.3	± 0.6	0.002	$2 \pm 0.005$	$-0.3 \pm 0.5$				

NC: Negative Control

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REMARKS	Filter	OPACITY
	1	A B
	2	A B
	3	A B

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Date 28-Feb-00

Test art	icle			Glycerol									
Batch N	0.			HS03116BS									
Concent	tration			100%		Treatme	time 10 min						
Code				B2	(4)								
Sequence				Intern 10	op-KIT								
				-									
No.	Treatment		Opacit	ty at			Permeability	7	In vitro sc	ore			
Comea		tO	t120	t120 - t0									
1	NC	0	0	0			0.004		0.1				
2	MEM	0	0	0			0.008		0.1				
3	100%	0	1	1			0.010		1.2				
	Mean ± S.D.			0.3	<b>±</b> 0.6		$0.007 \pm 0.0$	03	0.5 ±	0.6			
	_				Correcte	d value	Corr	ected value					
7	Test article	1	0	-1	-1.3		0.017 0.0	10	-1.2				
8		1	1	0	-0.3		0.015 0.0	08	-0.2				
9	100%	1	0	-1	-1.3		0.017 0.0	10	-1.2				
	Mean $\pm$ S.D.				-1.0	± 0.6	0.0	09 ± 0.001	-0.9 ±	0.6			

NC: Negative Control

REMARKS	Filter	OPACITY
	1	AB
	2	AB
	3	AB

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Date 31-Jan-00

Test art	icle			Methyl c	ethyl cyclopentane									
Batch N	lo.			09817PS-	089						_			
Concen	tration			100%	~	Treatme	ent time	10 min						
Code				D5	(5)					-				
Sequen	ce			12A					OP-KIT					
						-								
No.	Treatment		Opaci	ty at			Perm	eability	In v	itro score				
Comea		t0	t120	t120 - t0	]									
					-									
1	NC	0	0	0	1		0.004		0.1					
2	NaC10.9%	1	1	0	1		0.006		0.1					
3	100%	1	1	0	1		0.005		0.1					
	Mean ± S.D.			0.0	± 0.0	1	0.005	± 0.001	0.1	± 0.0	)			
		•				•								
					Correcte	ed value		Corrected value						
16	Test article	0	1	1	1.0		0.262	0.257	4.9					
17	1	0	2	2	2.0	1	0.839	0.834	14.5	5				
18	100%	0	0	0	0.0	1	0.212	0.207	3.1					
	Mean ± S.D.				1.0	± 1.0		$0.433 \pm 0.348$	3 7.5	± 6.1	_			
	$Mean \pm S.D.$				1.0	± 1.0		$0.433 \pm 0.348$	3 7.5	± (	5.1			

NC: Negative Control

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REMARKS	Filter	OPACITY
	1 🕅	
	2 A	В
	3 A	В

#### Paraph 20-Mar-00

Date

Test art	icle			Tween 2	0							
Batch N	lo.			A010055	102							
Concen	tration			100%		Treatm	ent time	10 min				
Code				C2	(6)							
Sequen	ce			11A		OP-KIT						
						_						
					_							
No.	Treatment		Opacit	ty at	1		Perm	eability	Г	In vitro	score	
Cornea		t0	t120	t120 - t0	]							
					-							
1	NC	0	0	0	]		0.002		Г	0.0		
2	NaC1 0.9%	0	0	0	]		0.003		Г	0.0		
3	100%	1	1	0	1		0.001		Г	0.0		
	Mean ± S.D.			0.0	± 0.0		0.002	± 0.001	Г	0.0	± 0	0.0
						-			-			
					Correcte	ed value		Corrected value				
7	Test article	0	0	0	0.0		0.010	0.008	[	0.1		
8		0	0	0	0.0	]	0.023	0.021	Г	0.3		
9	100%	0	0	0	0.0		0.004	0.002		0.0		
	Mean ± S.D.				0.0	± 0.0		0.010 ± 0.01	0	0.1	± 0	).2
						_ 0.0		0.010 0.01	Ľ.	0.1	_ 0	_

NC: Negative Control

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REMARKS	Filter	OPACITY
	1	AB
	2	AB
	3	AB

Paraph

Date 28-Feb-00

				1								
est art	icle			Methyl 18	so-butyl k	etone (4 m	ethyl-2-pent	anone) [	<u>108-10-1</u>			
<b>Batch</b> N	0.			CU 10369	BU							
Concent	tration			100%		Treatme	ent time	10	min			
Code				A2	(7)							
Sequence	Sequence Inte			Intern 8E	, •••			OP-KIT				
No.	Treatment		Opaci	ty at			Permea	ability		In vitro score		
Cornea		t0	t120	t120 - t0								
		L			1							
1	NC	2	2	0	1		0.006			0.1		
2	MEM	1	1	0	1		0.012			0.2		
3	100%	1	3	2	1		0.009			2.1		
	Mean ± S.D.		L	0.7	± 1.2		0.009 ±	0.003		$0.8 \pm 1.1$		
		ł				4				0.0		
					Correcte	ed value	Г	Correcte	d value			
7	Test article	1	8	7	6.3		1.909	1.900	a raide	34.8		
8	1	0	7	7	6.3		0.621	0.612		15.5		
9	100%	1	9	8	7.3	1	0.706	0.697		17.8		
	Mean ± S.D.			<b></b>	6.6	± 0.6		1.070	± 0.720	$22.7 \pm 10.5$		
		1										

NC: Negative Control

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REMARKS	Filter	OPACIT	Y
	1 2	A B	
	3	A B	

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Date 18-Jan-00

Test art	icle			Toluene	[108-88-3	31				
Batch N	0.			9902810	1100000000000000000000000000000000000	 				
Concen	tration			100%		Treatme	nt time			
Code				D4	(র্ম)					
Sequence 1			12A	<u> </u>	1			OP-	KIT	
										· · · · · · · · · · · · · · · · · · ·
No.	Treatment		Opaci	ty at	1		Perm	eability		In vitro score
Cornea		t0	t120	t120 - t0	1					
					-					
1	NC	0	0	0	1		0.004			0.1
2	NaC1 0.9%	1	1	0	1		0.006			0.1
3	100%	1	1	0	1		0.005			0.1
	Mean ± S.D.			0.0	± 0.0	1	0.005	± 0.001		$0.1 \pm 0.0$
						•				
					Correcte	ed value	]	Corrected va	alue	
13	Test article	0	6	6	6.0		3.480	3.475		58.1
14	1	0	7	7	7.0	1	2.832	2.827		49.4
15	100%	0	6	6	6.0	1	3.236	3.231		54.5
	Mean ± S.D.				6.3	± 0.6		3.178 ±	0.327	$54.0 \pm 4.4$
		•								

NC: Negative Control

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REMARKS	Filter OPACITY
	1 A B
	2 A B
	3 A B

Paraph

Date 20-Mar-00

200	fala			methyl a	mul ketor	e (? henter	$(n_{n}) [110]$	12 01	
1 est ai t	icie			111cury1 a	A RELOI		10110) [110		
Batchin	0.			1000	+		- t time	10 min	
Concem	Fation			100%		Treame	ntinne	10 min	
Code				A3	(9)			·····	
Sequence			Intern 8E	, •				OP-KIT	
No.	Treatment		Opacit	ty at	1		Perme	ability	In vitro score
Cornea		tO	t120	t120 - t0					
			<u></u>						
1	NC	2	2	0	1		0.006		0.1
2	MEM	1	$\frac{1}{1}$	0	1		0.012		0.2
3	100%	1	3	2			0.009		2.1
<u> </u>	Mean $+$ S.D.		<u> </u>	0.7	+ 1.2	1	0.009	0.003	0.8 + 1.1
	Mean 2 0.2	ł		0.7		1	0.007	0.000	
					Correct	d value	Г	Corrected value	
10	Test article	1		7	63		1.095		
10	Test article	1	0		5.2		1.095	1.060	22.0
11	1000	0	0	6	5.5		1.924	1.915	34.0
12	100%	2	/	5	4.5		2.404	2.395	40.2
	Mean $\pm$ S.D.				5.3	± 1.0	L	$1.799 \pm 0.663$	$2  32.3 \pm 8.9$

NC: Negative Control

REMARKS	Filter	Ol'ACITY	Y
	.1	A. B	
	2	A. B	
	3	A. B	

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Date 18-Jan-00

cle			2-methvl	-1-pentan	ol						
0.			05002PG	- pointeau							
ration			100%	-	Treatme	ent time 10 min					
			B3	(10)							
Sequence Intern 10A							OP-KIT				
Treatment		Opacit	ty at	1		Perm	eability	In vitro score			
	t0	t120	t120 - t0								
NC	0	0	0			0.004		0.1			
MEM	0	0	0			0.008		0.1			
100%	0	1	1			0.010		1.2			
Mean ± S.D.			0.3	<b>±</b> 0.6		0.007	± 0.003	$0.5 \pm 0.6$			
				Correcte	d value		Corrected value				
Test article	0	10	10	9.7		3.336	3.329	59.6			
	0	13	13	12.7		4.916	4.909	86.3			
100%	0	14	14	13.7		4.680	4.673	83.8			
Mean ± S.D.				12.0	<b>±</b> 2.1		4.304 ± 0.852	$76.6 \pm 14.7$			
	cle o. ration e Treatment NC MEM 100% Mean ± S.D. Test article 100% Mean ± S.D.	cle           0.           ration           e           Treatment           t0           NC         0           MEM         0           100%         0           Mean ± S.D.         0           Togs article         0           0         0           Mean ± S.D.         0	cle         o.         ration         e         Treatment       Opacit         t0       t120         NC       0       0         MEM       0       0         100%       0       1         Mean ± S.D.       100       13         Test article       0       14         Mean ± S.D.       14	cle       2-methyl         0.       05002PG         ration       100%         B3       B3         e       Intern 10         Treatment       Opacity at         t0       t120         NC       0       0         MEM       0       0         100%       0       1         Mean ± S.D.       0.3         Test article       0       10         0       13       13         100%       0       14         Mean ± S.D.       14       14	cle       2-methyl-1-pentan         0. $05002PG$ ration $100\%$ B3 $100\%$ e       Intern 10A         Treatment         Opacity at t0       t120         NC       0       0         MEM       0       0         100%       0       1         Mean ± S.D.       0.3       ±       0.6         Correcte         Test article       0       10       9.7         0       13       13       12.7         100%       0       14       14       13.7	cle       2-methyl-1-pentanol         0. $05002PG$ ration $100\%$ B3 $100\%$ e       Intern 10A         Treatment         Opacity at t0 $1120$ NC       0       0         MEM       0       0 $100\%$ 0       1         Mean $\pm$ S.D. $0.3 \pm 0.6$ Corrected value         Test article       0       10       9.7         0       13       13       12.7         100%       0       14       14       13.7	cle       2-methyl-1-pentanol         0.       05002PG         ration       100%         B3       (O)         e       Intern 10A         Perm         t0       t120       t120-t0         NC       0       0       0         MEM       0       0       0         100%       0       1       1         Mean ± S.D.       0.3       ±       0.6         Corrected value         Test article       0       10       10       9.7       3.336         100%       0       14       14       13.7       4.680	cle       2-methyl-1-pentanol         0.       05002PG         ration       100%         B3       Image: Constraint of the second			

NC: Negative Control

REMARKS	Filter	OPACITY
	1	A. B
	2	A. B
	3	A. B

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Eate 31-Jan-00

Test article       Ethanol [64-17-5]         Batch No.       9930710002         Concentration       100%         Code       D1       Image: Concentration of the second s					<b>T</b> .1 1 1	CA 45 51		_					
Batch No.         9930710002           Concentration         100%           Code         D1         Image: Concentration           Sequence         12A         Treatment time         10 min           Sequence         00         0         0         0         0           No. Cornea         Treatment         Opacity at t0         Permeability         In vitro score           1         NC         0         0         0         0         0         0           1         NC         0	lest arti	cle	· · · · · · · · · · · · · · · · · · ·		Ethanol	64-17-5]							
Concentration         100%         Treatment time         10 min           Code         D1         Mo.         Treatment         0P-KIT           Sequence         12A         Permeability         In vitro score           No.         Treatment         Opacity at t0         Permeability         In vitro score           1         NC         0         0         0         0         0.1         0.1           2         NaCl 0.9%         1         1         0         0.00         0.006         0.1         1.1         0         0.0         0.0         0.1         1.1         0.1         0.1         1.1         0.1	Batch No	).			9930710002								
Code         D1 $(14)$ Sequence         12A         OP-KIT           No.         Treatment         Opacity at         0         0           Comea         t0         t120         t120-t0         In vitro score           1         NC         0         0         0         0.1           2         NaCl 0.9%         1         1         0         0.004         0.1           3         100%         1         1         0         0.005         0.01         0.1           Mean $\pm$ S.D.         0.0 $\pm$ 0.0         0.005 $\pm$ 0.001         0.1 $\pm$ 0.1         0.1           4         Test article         0         16         16         16.0         2.340         2.335         51.0           4         100%         0         15         15         15.0         2.520         2.515         52.7	Concenti	ration			100%	(	Treatme	nt time	10 min				
Sequence         12A         OP-KIT           No. Cornea         Treatment         Opacity at t0         Permeability         In vitro score           1         NC         0         0         0         0.004         0.1           2         NaCl 0.9%         1         1         0         0.006         0.01         0.1           3         100%         1         1         0         0.005         0.001         0.1         1.1           Mean $\pm$ S.D.         0.0 $\pm$ 0.0         Corrected value         Corrected value         Corrected value         1.1	Code				D1	(11)							
No. Comea         Treatment         Opacity at t0         Permeability         In vitro score           1         NC         0         0         0         0.004         0.1           2         NaCl 0.9%         1         1         0         0.006         0.1           3         100%         1         1         0         0.005         0.01         0.1           Mean ± S.D.         0.0         ±         0.0         ±         0.001         0.1         ±           Corrected value         Corrected value           4         Test article         0         16         16         16.0         2.340         2.335         51.0           5         0         17         17         17.0         2.520         2.515         49.4	Sequence 12A OP-KIT					P-KIT							
No. Cornea         Treatment         Opacity at t0         Permeability         In vitro score           1         NC         0         0         0         0.004         0.1           2         NaCl 0.9%         1         1         0         0.006         0.1           3         100%         1         1         0         0.005         0.1           Mean $\pm$ S.D.         0.0 $\pm$ 0.0         0.005         0.001         0.1           Corrected value         Corrected value         Corrected value         0.1 $\pm$ 4         Test article         0         16         16         16.0         2.164         2.159         49.4           5         100%         0         15         15.0         15.0         2.515         52.7													
No. Cornea         Treatment         Opacity at t0         Permeability         In vitro score           1         NC         0         0         0         0.004         0.1           2         NaCl 0.9%         1         1         0         0.006         0.1           3         100%         1         1         0         0.005         0.1           Mean $\pm$ S.D.         0.0 $\pm$ 0.0         0.005 $\pm$ 0.001         0.1         1           Corrected value         Corrected value         Corrected value         0.1 $\pm$ 1           4         Test article         0         16         16         16.0         2.340         2.335         51.0           5         0         17         17         17.0         2.520         2.515         52.7				ı									
Cornea         t0         t120         t120-t0           1         NC         0         0         0           2         NaCl 0.9%         1         1         0           3         100%         1         1         0           Mean $\pm$ S.D.         0.0 $\pm$ 0.0 $0.005$ 0.005         0.01         0.1         0.1           Mean $\pm$ S.D.         0.0 $\pm$ 0.0 $0.005 \pm$ $6$ 100%         0         16         16         16.0           5         0         17         17         17.0         2.164         2.159           49.4         15         15         15.0         2.520         2.515         52.7	No.	Treatment		Opacit	ty at			Perm	eability	In vitro score			
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Cornea		t0	t120	t120 - t0								
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$						-							
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	1	NC	0	0	0			0.004		0.1			
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	2	NaC1 0.9%	1	1	0	1		0.006		0.1			
Mean $\pm$ S.D.         0.0 $\pm$ 0.0         0.005 $\pm$ 0.001         0.1 $\pm$ 4         Test article         0         16         16         16.0         2.340         2.335         51.0           5         0         17         17         17.0         2.164         2.159         49.4           6         100%         0         15         15         15.0         2.515         52.7	3	100%	1	1	0			0.005		0.1			
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		Mean ± S.D.			0.0	± 0.0		0.005	± 0.001	$0.1 \pm 0.0$			
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	-												
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$						Correcte	d value		Corrected value				
5         0         17         17         17.0         2.164         2.159         49.4           6         100%         0         15         15         150         2520         2515         527	4	Test article	0	16	16	16.0		2.340	2.335	51.0			
6 100% 0 15 15 15 2520 2515 527	5		0	17	17	17.0		2.164	2.159	49.4			
0 10070 0 15 15 15.0 2.520 2.515 52.7	6	100%	0	15	15	15.0		2.520	2.515	52.7			
Mean $\pm$ S.D. 16.0 $\pm$ 1.0 2.336 $\pm$ 0.178 51.0 $\pm$		Mean ± S.D.				16.0	<b>±</b> 1.0		$2.336 \pm 0.178$	51.0 ± 1.7			

NC: Negative Control

REMARKS	Filter	OPACITY	Y
	1	AB	
	2	A. B	
	3	A. B	

Paraph

Date 20-Mar-00

Test art	icle			Sodium	hydroxide	1%					
<b>Batch</b> N	lo.			66H0320							
Concen	tration			1%	$\overline{\mathbf{A}}$	Treatme	ent time				
Code				D3	(12)						
Sequence 12A			12A	$\overline{}$				OP-KI	Г		
No.	Treatment		Opaci	tv at	1		Perm	eability		In vitro s	core
Cornea		tO	t120	t120 - t0						III VIGO D	0010
1	NC	0	0	0	1		0.004	1		0.1	
2	NoCl 0.0%	1	1	0	1		0.004			0.1	
- 2	100%		1	0	-		0.000		- H	0.1	
	Mean + S D	1		0.0	<u>+ 00</u>		0.005	+ 0.001	- H-	0.1	0.0
	Weat $\pm 5.D$ .			0.0	± 0.0		0.005	± 0.001		<u>0.1</u> ±	. 0.0
					Correcte	1 value		Corrected value			
10	Test article	0	101	101	101.0		3.952	3.947		160.2	
11		0	111	111	111.0		4.276	4.271		175.1	
12	100%	0	87	87	87.0		4.256	4.251		150.8	
	Mean ± S.D.				99.7	± 12.1		4.156 ± 0.18	2	162.0 ±	: 12.3
							1				

NC: Negative Control

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REMARKS	Filter	OPACITY
	1	A B
	3	A B

Paraph

Date 20-Mar-00

					100 (50)					
Test art	icle			Triton X	-100 (5%)	)				
<b>Batch</b> N	lo.			28H2536		_				
Concent	tration			100% Treatment time 10 min						
Code				C4	(13)					
Sequence 1			11A			OP-KIT				
						•				
No.	Treatment		Opaci	tv at			Perm	eability		In vitro score
Cornea		t0	t120	t120 - t0				,		
					8					
	NC	0	0	0	1		0.002		1	0.0
2		0		0	1		0.002			0.0
2	100%	1		0	4		0.003			0.0
	100%	1		0	. 00		0.001	. 0.001		0.0
	Mean $\pm$ S.D.			0.0	± 0.0		0.002	$\pm 0.001$		$0.0 \pm 0.0$
					Correcte	ed value		Corrected value	,	
13	Test article	1	6	5	_ 5.0		4.268	4.266		69.0
14		0	4	4	4.0		3.384	3.382		54.7
15	100%	2	6	4	4.0		3.792	3.790		60.9
	Mean ± S.D.		•		4.3	± 0.6		$3.813 \pm 0.44$	42	$61.5 \pm 7.2$
		1								

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A. B	
	2	A. B	
	3	A. B	

Paraph

Date 28-Feb-00

Test art	icle			n-octano	1					
Batch N	0.			27336-01	9					
Concen	tration			100%	$\sim$	Treatm	ent time	10 min		
Code				B4	(14)					
Sequence				Intern 10					OP-I	KIT
No.	Treatment		Opacit	ty at			Perm	eability		In vitro score
Cornea		t0	t120	t120 - t0						
					-					
1	NC	0	0	0			0.004			0.1
2	MEM	0	0	0			0.008			0.1
3	100%	0	1	1		_	0.010			1.2
	Mean ± S.D.			0.3	± 0.6		0.007	± 0.003		$0.5 \pm 0.6$
			'						'	
					Correcte	ed value		Corrected value		
13	Test article	1	7	6	5.7		5.180	5.173		83.3
14		0	15	15	14.7	]	5.828	5.821		102.0
15	100%	1	11	10	9.7		4.724	4.717		80.5
	Mean ± S.D.				10.0	± 4.5		5.237 ± 0.	555	88.6 ± 11.7
		-								

NC: Negative Control

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REMARKS	Filter	OPACITY
	1 7	B
	2 /	B
	3 4	В

Paraph

Date 31-Jan-00

Test art	ticle			2-ethyl-1	-hexanol	[107-76-7]			
Batch N	lo.			26812-01	9				
Concen	tration			100%	$\sim$	Treatme	ent time	10 min	
Code				A4	(15)				
Sequence				Intern 8E		1			OP-KIT
						-		-	
No.	Treatment		Opaci	ty at			Perme	ability	In vitro score
Cornea		t0	t120	t120 - t0	1				
					•				
1	NC	2	2	0	]		0.006		0.1
2	мем	1	1	0	1		0.012		0.2
3	100%	1	3	2	1		0.009		2.1
	Mean ± S.D.			0.7	± 1.2	1	0.009 ±	0.003	$0.8 \pm 1.1$
					Correcte	ed value	Г	Corrected value	- <b>-</b>
13	Test article	2	6	4	3.3		1.763	1 754	29.6
14			7	6	53	1	2 196	2 187	38.1
15	100%	$\frac{1}{2}$	7	5	43	1	1 337	1 328	24.2
	Meen + SD		′		4.3	+ 10	1.557	1.520 1.756 ± 0.420	$20.6 \pm 7.0$
	$Mean \pm 3.D.$	1			4.3	± 1.0	L	1.730 ± 0.430	$50.0 \pm 7.0$

NC: Negative Control

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REMARKS	Filter	OPACITY
	1	A, B
	2	A. B
	3	A. B

Paraph

Date 18-Jan-00

Test art	icle			1-Hexan	ol [111-27-3	5]					
<b>Batch</b> N	0.			381949/1							
Concent	tration			100%		Treatme	nt time				
Code				D2	(16)						
Sequence			12A					OP-KI	Т	_	
No.	Treatment		Opacit	ty at	1		Perm	eability	Г	In vitro score	
Cornea		t0	t120	t120 - t0				-			
					•						
1	NC	0	0	0	]		0.004		Г	0.1	
2	NaC1 0.9%	1	1	0	1		0.006			0.1	
3	100%	1	1	0	1		0.005			0.1	
	Mean ± S.D.		L	0.0	± 0.0		0.005	± 0.001		0.1 + 0.0	
		I							L	0.1 2 0.0	
					Corrected	value		Corrected value			
7	Test article	0	17	17	17.0		3,700	3.695		72.4	
8	1	0	13	13	13.0		4 060	4 055		73.8	
9	100%	0	16	16	16.0		3,440	3.435		67.5	
<u> </u>	Mean + S D		10	L	15.3 +	21		$3728 \pm 0.31$		71.2 + 3.3	_
	intear ± 0.D.				15.5 ±	2.1		$5.720 \pm 0.31$	1	/1.2 <u>±</u> 3.3	_

NC: Negative Control

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REMARKS	Filter	OPACITY
	1	A B
	3	A B

Paraph

Date 20-Mar-00

	•			A	CT ( 4 1]				
lest art	icle			Acetone	[6/-64-1]				
Batch N	0.			<b>39H343</b> 0					
Concentration 100%						Treatme	nt time	10 min	
Code				A5	(17)				
Sequence	ce			Intern 8E				Ő	P-KIT
						1	i i		
No.	Treatment		Opaci	ty at			Permea	ability	In vitro score
Comea		tO	t120	t120 - t0				,	
1	NC	2	2	0	1		0.006		0.1
1		2		0			0.000		0.1
2	MEM	1	1	0			0.012		0.2
3	100%	1	3	2			0.009		2.1
	Mean $\pm$ S.D.			0.7	± 1.2		0.009 ±	0.003	$0.8 \pm 1.1$
		•				•			
					Correcte	d value	Г	Corrected value	7
16	Test article	1	36	35	34.3		1.688	1.679	59.5
17	1	1	42	41	40.3		2.888	2.879	83.5
18	100%	1	44	43	42.3		4.304	4.295	106.7
	Mean ± S.D.				39.0	± 4.2		$2.951 \pm 1.309$	83.2 ± 23.6
							_		_

NC: Negative Control

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REMARKS	Filter OPACITY
	1 A B
	2 A B
	3 A B

Paraph

Date 18-Jan-00

Test art	icle			Cyclohex	anol						
<b>Batch</b> N	0.			18285-049	)						
Concent	tration			100%		Treatme	ient time 10 min				
Code				B5	(18)						
Sequence	e			Intern 10	A			OP-F	<u>KIT</u>		
			_		i						
No.	Treatment		Opacit	ty at			Permeability		In vitro score		
Cornea		t0	t120	t120 - t0							
					-						
1	NC	0	0	0			0.004		0.1		
2	MEM	0	0	0			0.008		0.1		
3	100%	0	1	1		_	0.010		1.2		
	Mean ± S.D.			0.3	± 0.6		$0.007 \pm 0.003$		$0.5 \pm 0.6$		
		-				-					
					Correcte	ed value	Corrected va	lue			
16	Test article	0	16	16	15.7		6.180 6.173		108.3		
17		0	16	16	15.7		3.288 3.281		64.9		
18	100%	0	14	14	13.7		5.680 5.673		98.8		
	Mean $\pm$ S.D.				15.0	± 1.2	$5.042 \pm$	1.546	$90.7 \pm 22.8$		

NC: Negative Control

REMARKS	Filter	OPACITY
	1	A. B
	2	A. B
	3	A. B

Peraph

Eate 31-Jan-00

Tost ort	iele			Cetvlpvr	idinium b	romide (6)	76)					
Roteh N	o			10500014			///)					
Concept	U, Tration			1001091	,	Treatm	ent time					
Code	ANDON			100%	$\overline{(n)}$	Teann						
Code				114	5		0.0 111					
Sequence	e.			IIA					OP			
No.	Treatment		Opacit	y at	1		Permea	ability		In vitro score		
Cornea		t0	t120	t120 - t0								
1	NC	0	0	0	1		0.002			0.0		
2	NaC1 0.9%	0	0	0	1		0.003			0.0		
3	100%	1	1	0	1		0.001			0.0		
	Mean ± S.D.			0.0	± 0.0		0.002 ±	0.001		$0.0 \pm 0.0$		
		1										
					Correcte	d value	Г	Corrected valu	le			
16	Test article	1	15	14	14.0		1.659	1.657	······	38.9		
17		1	12	11	11.0		0.657	0.655		20.8		
18	100%	0	10	10	10.0		0.714	0.712		20.7		
	Mean ± S.D.				11.7	± 2.1		$1.008 \pm 0$	0.563	26.8 + 10.5		

NC: Negative Control

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REMARKS	Filter	OPACITY
	1	AB
	2	A B
	3	A B

Paraph

Date 28-Jan-00

# Benzalkonium chloride (10%)

Exp.	Opacity	Permeability	In Vitro Score
1	88.0	4.426	154.4
2	94.6	4.148	156.9
3	87.0	4.252	150.8
4	93.0	4.278	157.2
5	98.3	3.972	157.9
mean	92.2	4.2	155.4
SD	4.7	0.17	2.9

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(<sup>\*</sup> )

#### **BCOP PREVALIDATION 1997**

#### Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article 1 (BAK)				
Batch No. 76H2520				
Concentration 10 g/g%		Treatment time	10	min
Prevalidation phase II	· .			
Sequence				

					-						
No.	Treatment	(	Opacity	at		Permeability			In vitro	In vitro score	
Cornea		t0	t120	t120 - t0							
				-				-			
16	NC	1	2	1			0.005			1.1	
17	0.9 % NaCl	1	2	1			0.018			1.3	
18	100%	1	2	1		_	0.002			1.0	
	Mean ± S.D.			1.0	± 0.0		$0.008 \pm 0.009$		1.1 :	± 0.2	
						•				-	
					Corrected	value		Corrected	value		
19	Test article	1	97	96	95.0		3.927	3.919		153.8	
20		1	82	81	80.0		4.245	4.237		143.6	
21	10g/g%	1	91	90	89.0		5.130	5.122		165.8	
	Mean ± S.D.				88.0	± 7.5		4.426	± 0.623	154.4 :	± 11.1

NC: Negative control

PC: Positive control

REMARKS	Filter OPACITY	
	1 A 75 B -	75
	2 A 153 B -1	59
	3 A 236 B -2	53

Paraph

Date 13-Feb-97

## **BCOP PREVALIDATION 1997**

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	
Batch No. 76H2520	
Concentration 10 g/g%	Treatment time 10 min
Prevalidation phase II	
Sequence D	

					-						
No.	Treatment	0	Opacity at				Permeability			In vitro score	
Cornea		t0	t120	t120 - t0							
16	NC	1	1	0			0.005			0.1	
17	0.9 % NaCl	1	2	1			0.007			1.1	
18	100%	0	1	1		_	0.004			1.1	
	Mean ± S.D.			0.7	± 0.6		0.005	± 0.002		0.8	± 0.6
					Corrected	value		Corrected val	ue		
19	Test article	1	108	107	106.3		4.785	4.780		178.0	
20		0	92	92	91.3		3.464	3.459		143.2	
21	10g/g%	0	87	87	86.3		4.210	4.205		149.4	
	Mean ± S.D.				• 94.6	± 10.4		4.148 ±	0.662	156.9	± 18.6
		-									

NC: Negative control

PC: Positive control

REMARKS	Filter OPACITY
	1 A 75 B -75
	2 A 153 B -158
	3 A 235 B -252

#### Paraph

Date

#### **BCOP PREVALIDATION 1997**

#### Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test articles 1 (BAK)	
Batch No. 76H2520	
Concentration 10 g/g%	Treatment time 10 min
Prevalidation phase	
Sequence	

No.	Treatment	Opacity at			Perm		eability		In vitro sco	ore	
Cornea		t0	t120	t120 - t0							
								•			
10	NC	0	1	1			0.009			1.1	
11	0.9 % NaCl	1	2	1			0.001			1.0	
12	100%	0	1	1			0.018			1.3	
	Mean ± S.D.			1.0	± 0.0		0.009	± 0.009		1.1 ±	0.2
									<del></del>		
					Corrected	value		Corrected	value		
19	Test article	1	88	87	86.0		4.333	4.324		150.9	
20		1	82	81	80.0		4.255	4.246		143.7	
21	10g/g%	1	97	96	95.0		4.196	4.187		157.8	
	Mean ± S.D.				87.0	± 7.5		4.252	± 0.069	150.8 ±	7.1

NC: Negative control

PC: Positive control

REMARKS	Filter OPACITY
	1 A 75 B -75
	2 A 152 B -158
······································	3 A 234 B -252

Paraph

Date 13-Mar-97

## **BCOP PREVALIDATION 1997**

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	1 (BAK)		
Batch No.	76H2520		
Concentration	10 g/g%	Treatment time	10 min
Prevalidation phase	II		
Sequence	G		

					_						
No.	Treatment	(	Opacity	at			Perm	eability		In vitro sco	ore
Cornea		t0	t120	t120 - t0							
19	NC	3	3	0			0.008			0.1	
20	0.9 % NaCl	0	0	0			0.038			0.6	
21	100%	1	1	0		_	0.012		_	0.2	
	Mean ± S.D.			0.0	± 0.0		0.019	± 0.016		0.3 ±	0.3
						-			•		
							1				
					Corrected	value		Corrected	value		
25	Test article	0	96	96	96.0		4.531	4.512		163.7	
26		0	93	93	93.0		5.219	5.200		171.0	
27	10g/g%	2	92	90	90.0		3.142	3.123		136.8	
	Mean ± S.D.				93.0	± 3.0		4.278	± 1.058	157.2 ±	18.0

NC: Negative control

PC: Positive control

REMARKS	Filter	OPACITY	Y
	1 A	75 B	-75
	2 A	152 B	-158
	3 A	231 B	-249

Paraph

Date 20-Mar-97

## **BCOP PREVALIDATION 1997**

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article 1 (BAK)	
Batch No. 76H2520	
Concentration. 10 g/g%	Treatment time 10 min
Prevalidation phase II	
Sequence F H	

					-		_				
No.	Treatment		Opacity	at			Perm	eability	-	In vitro s	core
Cornea		t0	t120	t120 - t0							
10	NC	1	1	0			0.013			0.2	
11	0.9 % NaCl	1	4	3			0.013			3.2	
12	100%	1	1	0		_	0.021			0.3	
	Mean ± S.D.			1.0	± 1.7		0.016	± 0.005		1.2 ±	- 1.7
					Corrected	value		Corrected	value		
19	Test article	1	99	98	97.0		4.051	4.035		157.5	
20		1	99	98	97.0		4.312	4.296		161.4	
21	10g/g%	1	103	102	101.0		3.600	3.584		154.8	
	Mean ± S.D.				98.3	± 2.3		3.972	± 0.360	157.9 ±	3.3

NC: Negative control

PC: Positive control

REMARKS	Filter OPACITY
	1 A 75 B -75
	2 A 152 B -161
	3 A 236 B -253

Paraph

Date 21-Mar-97

# Compounds 1 → 20 young animals (6-8 monHs)

#### VALIDATION

#### Calculation of the in vitro eye irritation score for liquids

Test article 3,3 Dimethylpentane [562-49-2]									
Batch No.	14602CN								
Concentration	99%	Treatment time	10	min					
Code	A1 1								
Sequence	2005/ Intern3 kalv	eren			OP-KIT				



NC: Negative Control

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REMARKS			Filter	OPACIT	ITY	
			1 A 2 A 3 A	75 B 158 B 256 B	-75 -160 -258	
	Filter					
Paraph	0.1	1				
	0.3	15				
Date 07-Mar-05	0.6	50				
	0.8	90				
	1	145		RDF/B	8CO/18	

#### Calculation of the in vitro eye irritation score for liquids **Test article** 3-methoxy-1,2-propanediol [623-39-2] Batch No. A0155893001 Concentration 100% Treatment time 10 min Ê Code B1 2005/ intern2 kalveren Sequence **OP-KIT** No. Treatment Opacity at Permeability In vitro score t120 t120 - t0 t0 Comea 1 NC 0 0 0 0.084 1.3 2 NaC1 0.9% 0 2 2 0.085 3.3 100% 3 0 0.036 1 1 0.5 Mean ± S.D. 0.7 ± 1.2 0.068 ± 0.028 1.7 1.4 ± Corrected value Corrected value 0 0 0.090 4 Test article 0 -0.7 0.022 -0.4 5 0.028 2 6 4 3.3 0.096 3.7 0 0 6 100% 0 -0.7 0.070 0.002 -0.7 Mean ± S.D. 0.6 ± 2.3 0.017 ± 0.014 0.9 2.5 ±

NC: Negative Control

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REMARKS			 Filter OPACITY			
			1 A 2 A 3 A	75 B 155 B 259 B	-75 -161 -261	
	Filter					
Paraph	0.1	1				
	0.3	16				
Date 01-Mar-05	0.6	51				
	0.8	91				
	1	143		RDF/B	CO/18	

#### Calculation of the in vitro eye irritation score for liquids polyethylene glycol 400 [25322-68-3] Test article Batch No. S23152-394 Concentration 100% Treatment time 10 min C1 (3) 2005/ intern 1 kalveren Code **OP-KIT** Sequence No. Treatment Opacity at Permeability In vitro score t0 t120 t120 - t0 Cornea NC 0 0 0.023 0 1 0.3 NaCl 0.9% 0 0 0.069 2 0 1.0 3 100% 0 0 0 0.044 0.7 0.0 0.045 ± 0.023 0.7 Mean ± S.D. ± 0.0 0.4 ± Corrected value Corrected value 0 0 0 0.0 0.102 0.057 0.9 4 Test article 5 0 0 0 0.0 0.178 0.133 2.0 6 100% 0 0 0 0.0 0.080 0.035 0.5 Mean ± S.D. 0.0 0.0 0.075 ± 0.051 0.8 ± 1.1 ±

NC: Negative Control

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REMARKS			Filter	OPACIT	Y
			1 A 2 A 3 A	75 B 157 B 260 B	-75 -161 -259
	Filter				
Paraph	0.1	1			
	0.3	16			
Date 28-Feb-05	0.6	50			
	0.8	88			
	1	140		RDF/B	CO/18

#### Calculation of the in vitro eye irritation score for liquids

Test article	glycerol [56	-81-5]		
Batch No.	13574HC			
Concentration	100%	Treatment time	10 min	
Code	B2 (4	,)		
Sequence	2005/ intern2	kalveren		OP-KIT
Sequence	2005/ 11101112	Karveren	·	
No Treatment	Opacity at	D	Permenhility	In vitro score



NC: Negative Control

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REMARKS			Filter OPACITY				
			1 A 2 A 3 A	75 B 155 B 259 B	-75 -161 -261		
	Filter						
Paraph	0.1	1					
	0.3	16					
Date 01-Mar-05	0.6	51					
	0.8	91					
	1	143		RDF/B	CO/18		

# Calculation of the in vitro eye irritation score for liquids

Test article	Methyl cyclopenta	ine [96-37-7]				
Batch No.	1097605					
Concentration	95%	Treatment time	10	min		
Code	D5 (5)					
Sequence	2005/ Intern3 kalv	veren			OP-KIT	



NC: Negative Control

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REMARKS			Filter	OPACIT	Ϋ́	
			1 A 2 A 3 A	75 B 158 B 256 B	-75 -160 -258	
	Filter					
Paraph	0.1	1				
	0.3	15				
Date 07-Mar-05	0.6	50				
	0.8	90				
	1	145		RDF/B	CO/18	

#### Calculation of the in vitro eye irritation score for liquids

0

0

0

0

0

0

0

0

0

0

0

0

0

0

0

0.0

0

0

0

± 0.0

Corrected value

±

0.0

0.0

0.0

0.0

Test artic	cle	Tween 20 {9005	5-64-5}		
<b>Batch</b> No	).	094K01761			
Concent	ration	100%	Treatment time	10 min	
Code		C2 (6)			
Sequence	e	2005/Intern4 kal	verogen		OP-KIT
			<u> </u>		
No.	Treatment	Opacity at	Perme	ability	In vitro score

0.045

0.022

0.012

0.026

0.028

0.021

0.013

**±** 0.017

0.002

-0.005

-0.013

-0.005 ±

0.008

Corrected value

0.7

0.3

0.2

0.4

0.0

-0.1

-0.2

-0.1

0.3

0.1

±

±



NC

NaC1 0.9%

100%

Mean ± S.D.

Test article

100%

Mean ± S.D.

1

2

3

19

20

21

REMARKS			Filter	OPACIT	Y
			1 A 2 A	75 B	-75 -158
			3 A	263 B	-258
	Filter				
Paraph	0.1	0			
	0.3	15			
Date 14-Mar-05	0.6	50			
	0.8	89			
	1	141		RDF/B	CO/18

0.0

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#### Calculation of the in vitro eye irritation score for liquids

Test article	Methyl iso-butyl k	Aethyl iso-butyl ketone (4 methyl-2-pentanone) [108-10-1]								
Batch No.	1127250									
Concentration	100%	Treatment time	10	min						
Code	A2 (7)									
Sequence	2005/ intern1 kalv	eren		OP-KIT						



NC: Negative Control

REMARKS			Filter OPACITY			
			1 A	75 B	-75	
			2 A 3 A	157 В 260 В	-161 -259	
	Filter					
Paraph	0.1	1				
	0.3	16				
Date 28-Feb-05	0.6	50				
	0.8	88				
	1	140		RDF/B	CO/18	

# Calculation of the in vitro eye irritation score for liquids

Test art	icle			Toluene	[108-88	3-3]			
<b>Batch</b> N	0,			A0204558	001				
Concentration 100%			100%	<u>~</u>	Treatment time	10	min		
Code D4			D4	(8)					
Sequence	e			2005/ Inte	em3 kalv	veren			OP-KIT
No.	Treatment	+0	Opacit	y at		Perme	ability		In vitro score
Comea		10	1120	1120-10					
1	NC	0	0	0		0.008			0.1
2	NaCl 0.9%	0	0	0		0.026			0.4
3	100%	0	0	0		0.006			0.1

	Mean ± S.D.			0.0	± 0.0		0.013	$0.013 \pm 0.011$			±	0.2
					Correcte	d value		Correcte	d value		_	
13	Test article	0	2	2	2.0		1.550	1.537		25.1		
14		0	9	9	9.0		1.852	1.839		36.6		
15	100%	0	7	7	7.0		1.030	1.017		22.3		
	Mean ± S.D.				6.0	± 3.6		1.464	<b>±</b> 0.416	28.0	±	7.6

NC: Negative Control

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REMARKS			Filter OPACITY			
			1 A 2 A 3 A	75 B 158 B 256 B	-75 -160 -258	
	Filter					
Paraph	0.1	1				
	0.3	15				
Date 07-Mar-05	0.6	50				
	0.8	90				
	1	145		RDF/B	CO/18	

#### Calculation of the in vitro eye irritation score for liquids

Test article         methyl amyl ketone (2 heptanone) [110-43-0]										
Batch No. 1362	2JC									
Concentration 1009	% Treatment time	10 min								
Code A3	(9)									
Sequence 2005	5/ intern 1 kalveren	OP-KIT								



NC: Negative Control

REMARKS			Filter	Filter OPACITY		
			1 A 2 A 3 A	75 B 157 B 260 B	-75 -161 -259	
	Filter					
Paraph	0.1	1				
	0.3	16				
Date 28-Feb-05	0.6	50				
	0.8	88				
	1	140		RDF/B	CO/18	

#### Calculation of the in vitro eye irritation score for liquids

Test article 2-methyl-1-pentanol [105-30-6]										
Batch No.	451942/1									
Concentration	100%	Treatment time	10	min						
Code	B3 (10)									
Sequence	2005/ intern2 kalv	eren			<b>OP-KIT</b>					



NC: Negative Control

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REMARKS			Filter	OPACIT	ITY	
			1 A 2 A	75 B 155 B	-75 -161	
Paraph	Filter 0.1	1	3_IA	259 8	-261	
Date 01-Mar-05	0.3 0.6 0.8	16 51 91				
	1	143		RDF/B	CO/18	

# Calculation of the in vitro eye irritation score for liquids

Test art	icle			Ethanol	[64-17-5	]			
<b>Batch</b> N	0.			K3395758	83 448	_			
Concent	ration			100%	$\sim$	Treatm	ent time	10 min	
Code				D1	(11)				
Sequence 2005/ In				tern3 kalv	eren		0	P-KIT	
			0		1		- D	1.111	T
No.	Treatment		Opaci	y at	4		Perm	eability	In vitro score
Comea		ťŪ	1120	t120 - t0					
			<u> </u>		,			1	
1	NC	0	0	0	1		0.008		0.1
2	NaC10.9%	0	0	0			0.026		0.4
3	100%	0	0	0			0.006		0.1
	Mean $\pm$ S.D.			0.0	± 0.0		0.013	± 0.011	$0.2 \pm 0.2$
					Correcte	d value		Corrected value	]
7	Test article	0	18	18	18.0		2.308	2.295	52.4
8		0	16	16	16.0		1.702	1.689	41.3
9	100%	0	15	15	15.0		1.531	1.518	37.8
	Mean ± S.D.				16.3	± 1.5		1.834 ± 0.408	43.8 ± 7.6

NC: Negative Control

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REMARKS			Filter	OPACIT	Y
			1 A 2 A 3 A	75 B 158 B 256 B	-75 -160 -258
	Filter				
Paraph	0.1	1			
	0.3	15			
Date 07-Mar-05	0.6	50			
	0.8	90			
	1	145		RDF/B	CO/18

Calcul	Calculation of the in vitro eye irritation score for liquids										
Test article       Sodium hydroxide 1% [1310-73-2]         Batch No.       014K0006         Concentration       1%         Treatment time       10       min         Code       D3       12         Sequence       2005/Intern4 kalverogen       OP							OP-H	<u>KIT</u>			
			0					141		T	
NO. Comea	Treatment	tO	t120	t120 - t0			Permeal	bility		lin Vitro	score
1 2 3	NC NaCl 0.9% 100% Mean ± S.D.	0 0 0	0 0 0	0 0 0 0.0	± 0.0		0.045 0.022 0.012 0.026 ±	0.017		0.7 0.3 0.2 0.4	± 0.3
16 17 18	Test article	0 0 0	139 145 123	139 145 123	Corrected 139.0 145.0 123.0	value	4.540 2.600 4.164	Corrected value 4.514 2.574 4.138	2	206.7 183.6 185.1	
	Mean ± S.D.				135.7 ±	11.4		3.742 ± 1.	.029	191.8	± 12.9

NC: Negative Control

REMARKS			 Filter OPA		
			1 A 2 A 3 A	75 B 156 B 263 B	-75 -158 -258
	Filter				
Paraph	0.1	0			
	0.3	15			
Date 14-Mar-05	0.6	50			
	0.8	89			
	1	141		RDF/B	CO/18

#### Calculation of the in vitro eye irritation score for liquids **Test article** Triton X-100 (5%) [9002-93-1] Batch No. A019437801 Treatment time Concentration 5% 10 min (13) C4 Code 2005/Intern4 kalverogen **OP-KIT** Sequence No. Treatment Opacity at Permeability In vitro score t0 t120 t120 - t0 Cornea



NC: Negative Control

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REMARKS			 Filter OPACI		
			1 A 2 A 3 A	75 B 156 B 263 B	-75 -158 -258
	Filter				
Paraph	0.1	0			
	0.3	15			
Date 14-Mar-05	0.6	50			
	0.8	89			
	1	141		RDF/B	CO/18

#### Calculation of the in vitro eye irritation score for liquids

Test article	a-octanol [111-8]	7-5]			
Batch No.	S02961-454				
Concentration	100%	Treatment time	10	min	
Code	B4 (14)				
Sequence	2005/ intern2 kalv	eren			OP-KIT



NC: Negative Control

REMARKS			Filter OPACITY		
			1 A 2 A 3 A	75 B 155 B 259 B	-75 -161 -261
	Filter				
Paraph	0.1	1			
	0.3	16			
Date 01-Mar-05	0.6	51			
	0.8	91			
	1	143		RDF/B	CO/18

# Calculation of the in vitro eye irritation score for liquids

Test article	2-ethyl-1-hexanol	[107-76-7]				
Batch No.	S01263-011					
Concentration	100%	Treatment time	10	min		
Code	A4 (15)					
Sequence	2005/ intern T kal	veren			OP-KIT	
					8 10 m	



NC: Negative Control

REMARKS			Filter	OPACIT	Ϋ́
			1 A 2 A 3 A	75 B 157 B 260 B	-75 -161 -259
	Filter				
Paraph	0.1	1			
	0.3	16			
Date 28-Feb-05	0.6	50			
	0.8	88			
	1	140		RDF/E	BCO/18

#### Calculation of the in vitro eye irritation score for liquids

01
Treatment time 10 min
16)
m3 kalveren OP-KIT



NC: Negative Control

REMARKS			Filter	OPACIT	ſY	
			1 A 2 A 3 A	75 B 158 B 256 B	-75 -160 -258	
	Filter					
Paraph	0.1	1				
	0.3	15				
Date 07-Mar-05	0.6	50				
	0.8	90				
	1	145		RDF/B	CO/18	

# Calculation of the in vitro eye irritation score for liquids

Test art Batch N Concent Code Sequenc	icle o. tration			Acetone 442942/1 100% A5 2005/ inte	[67-64-1] (17) ern 1 kalve	Treatme	nt time	10 min	OP-KIT	
No. Comea	Treatment	t0	Opacit t120	ty at t120 - t0			Perme	eability	In vitro sc	ore
1 2 3	NC 100% Mean ± S.D.	0 0 0	0 0 0	0 0 0 0.0	± 0.0		0.023 0.069 0.044 0.045	± 0.023	0.3 1.0 0.7 0.7 ±	0.4
16 17 18	Test article	0 0 0	101 92 81	101 92 81	Corrected 101.0 92.0 81.0	value	2.824 2.452 3.428	Corrected value 2.779 2.407 3.383	142.7 128.1 131.7	
	Mean ± S.D.				91.3	<b>±</b> 10.0		<b>2.856 ±</b> 0.493	3 134.2 <b>±</b>	7.6

NC: Negative Control

REMARKS			 Filter	OPACIT	Y
			1 A 2 A 3 A	75 B 157 B 260 B	-75 -161 -259
	Filter			2000	
Paraph	0.1	1			
	0.3	16			
Date 28-Feb-05	0.6	50			
	0.8	88			
	1	140		RDF/B	CO/18

Calcul	ation of the	e in v	itro e	eye irrita	tion sco	ore for li	quids			
Test art	icle			cyclohex	anol					
<b>Batch</b> N	0.			S05238-04	14					
Concent	tration			100%	$\sim$	Treatme	ent time	10 min		_
Code				B5	(18)					
Sequence	e.			2005/ inte	em2 kalve	eren		O]	P-KIT	
No.	Treatment		Opaci	ty at			Perm	eability	In vitr	o score
Comea		t0	t1 <b>2</b> 0	t1 <b>2</b> 0 - t0						
1	NC	0	0	0			0.084		1.3	
2	NaC1 0.9%	0	2	2			0.085		3.3	
3	100%	1	1	0			0.036		0.5	
	Mean ± S.D.			0.7	± 1.2		0.068	$\pm 0.028$	1.7	± 1.4
1										
1					Correcte	d value		Corrected value	]	
16	Test article	1	13	12	11.3		1.768	1.700	36.8	]
17	1	0	12	12	11.3		1.892	1.824	38.7	1
18	100%	0	13	13	12.3		2.940	2.872	55.4	1
	Mean ± S.D.			·	11.6	± 0.6	·	$2.132 \pm 0.644$	43.6	± 10.2

NC: Negative Control

REMARKS			Filter	OPACIT	FY	
			1 A 2 A 3 A	75 B 155 B 259 B	-75 -161 - <b>2</b> 61	
	Filter					
Paraph	0.1	1				
	0.3	16				
Date 01-Mar-05	0.6	51				
	0.8	91				
	1	143		RDF/B	CO/18	

#### Calculation of the in vitro eye irritation score for liquids

Test article	Cetylpyridinium b	etylpyridinium bromide (6%) [140-72-7]									
Batch No.	038H2509										
Concentration	6%	Treatment time	10	min							
Code	C5 (19')										
Sequence	2005/Intern4 kalve	erogen			<b>OP-KIT</b>						



NC: Negative Control

REMARKS			 Filter	OPACIT	ITY	
			1 A	75 B	-75	
			2 A 3 A	156 B 263 B	-158 -258	
	Filter					
Paraph	0.1	0				
	0.3	15				
Date 14-Mar-05	0.6	50				
	0.8	89				
	1	141		RDF/B	CO/18	

#### Calculation of the in vitro eye irritation score for liquids **Test article** Benzalkoniumchloride [8001-54-5] Batch No. 033K2544 Concentration 10g/g% Treatment time 10 min Code 20 C3 2005/Intern4 kalverogen Sequence **OP-KIT** No. Treatment Opacity at Permeability In vitro score t0 t120 t120 - t0 Cornea

					7		1			
1	NC	0	0	0		0.045			0.7	
2	NaCl 0.9%	0	0	0		0.022			0.3	
3	100%	0	0	0		0.012			0.2	
	Mean $\pm$ S.D.	í —		0.0	± 0.0	0.026	± 0.017		0.4 ±	0.3
			-							
					Corrected value	1	Corrected value			
7	Test article	0	115	115	115.0	4.016	3.990		174.9	
8	1 7	0	95	95	95.0	3.856	3.830		152.5	
9	100%	0	107	107	107.0	4.356	4.330		172.0	
-		-				4 · · · · · · · · · · · · · · · · · · ·				
-	Mean $\pm$ S.D.	<b>├</b>	<u> </u>		$105.7 \pm 10.1$		$4.050 \pm 0.2$	255	$166.5 \pm$	12.2

NC: Negative Control

REMARKS			Filter		OPACIT	Ϋ́Υ
			1	A	75 B	-75
			2	Α	156 B	-158
			3	A	263 B	-258
	Filter					
Paraph	0.1	0				
	0.3	15				
Date 14-Mar-05	0.6	50				
	0.8	89				
	1	141			RDF/E	BCO/18