

**Intralaboratory CV Analysis of BCOP -
Data from Dr. Freddy Van Goethem**

Substance	Concentration	Cornea number	Opacity	Permeability	<i>In Vitro</i> Score ¹	<i>In Vitro</i> Classification
2-Ethoxyethanol	100%	1	61.3	1.444	83	
		2	64	1.498	86.5	
		3	60	1.666	85	
		4	64	1.304	83.6	
		5	61.3	1.534	84.3	
		6	59.7	1.646	84.3	
		mean	61.717	1.515	84.450	Severe
		s.d.	1.886	0.134	1.214	
	%CV	3.1%	8.9%	1.4%		
Cyclohexanone	100%	1	80.8	3.954	140.1	
		2	76.2	5.393	157.1	
		3	70.2	4.373	135.8	
		4	75.5	3.903	134	
		5	80.5	4.081	141.7	
		6	76.5	4.341	141.6	
		mean	76.617	4.341	141.717	Severe
		s.d.	3.878	0.551	8.171	
	%CV	5.1%	12.7%	5.8%		
Gluconolactone	20%	1	88.7	0.171	91.2	
		2	86.7	0.181	89.4	
		3	78	0.143	80.1	
		4	78.7	0.21	81.8	
		5	88	0.1	89.5	
		6	91.3	0.118	93.1	
		mean	85.233	0.154	87.517	Severe
		s.d.	5.543	0.041	5.290	
	%CV	6.5%	26.8%	6.0%		
2,4-Pentanedione	100%	1	44.3	0.075	45.5	
		2	47.3	0.046	48	
		3	50.7	0.133	52.7	
		4	48.3	0.123	50.2	
		5	54.3	0.055	55.2	
		6	49.3	0.074	50.4	
		mean	49.033	0.084	50.333	Moderate
		s.d.	3.363	0.036	3.409	
	%CV	6.9%	42.4%	6.8%		
Promethazine hydrochloride	20%	1	119	0.261	122.9	
		2	134.3	0.564	142.8	
		3	144	0.051	144.8	
		4	140.7	0.539	148.8	
		5	143	0.179	145.7	
		6	128.7	0.128	130.6	
		mean	134.950	0.287	139.267	Severe
		s.d.	9.733	0.216	10.182	
	%CV	7.2%	75.3%	7.3%		
Deoxycholic acid, sodium salt	10%	1	12.2	5.181	89.9	
		2	14.8	5.49	97.2	
		3	11.8	6.097	103.3	
		4	16.8	5.204	94.9	
		5	16.5	6.444	113.2	
		6	10.8	5.895	99.3	
		mean	13.817	5.719	99.633	Severe
		s.d.	2.563	0.511	8.008	
	%CV	18.6%	8.9%	8.0%		
Furan	100%	1	24	1.886	52.3	
		2	22.7	2.274	56.8	
		3	19.7	1.815	46.9	
		4	19.3	1.771	45.9	
		5	21	1.93	49.9	
		6	17	2.143	49.1	
		mean	20.617	1.970	50.150	Moderate
		s.d.	2.513	0.197	3.966	
	%CV	12.2%	10.0%	7.9%		
Benzethonium chloride	10%	1	83.7	6.937	187.7	
		2	87.7	4.716	158.4	
		3	85.7	4.452	152.5	

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		4	83.7	5.911	172.3	
		5	79	4.769	150.5	
		6	88	5.736	174	
		mean	84.633	5.420	165.900	Severe
		s.d.	3.327	0.949	14.524	
		%CV	3.9%	17.5%	8.8%	
Hexadecyltrimethylammonium	10%	1	17.7	3.159	65.1	
		2	18.7	3.957	78	
		3	24.7	2.627	64.1	
		4	13.7	3.438	65.2	
		5	18.7	3.271	67.7	
		6	16.7	4.176	79.3	
		mean	18.367	3.438	69.900	Severe
		s.d.	3.615	0.562	6.893	
		%CV	19.7%	16.3%	9.9%	
Quinacrine	20%	1	52.3	0.024	52.7	
		2	58	0.041	58.6	
		3	64	0.097	65.4	
		4	53.3	0.018	53.6	
		5	51.7	0.085	52.9	
		6	62.7	0.112	64.3	
		mean	57.000	0.063	57.917	Severe
		s.d.	5.410	0.040	5.800	
		%CV	9.5%	63.9%	10.0%	
1-Nitropropane	100%	1	18.5	-0.002	18.5	
		2	14.5	0.002	14.5	
		3	16.8	-0.001	16.8	
		4	18.5	0.045	19.2	
		5	15.5	0.004	15.6	
		6	15.2	0	15.2	
		mean	16.500	0.008	16.633	Mild
		s.d.	1.719	0.018	1.885	
		%CV	10.4%	228.2%	11.3%	
Octanol	100%	1	19.2	2.582	57.9	
		2	32.5	1.946	61.7	
		3	28.2	2.265	62.1	
		4	31.2	2.685	71.4	
		5	30.5	2.091	61.9	
		6	24.8	1.7	50.3	
		mean	27.733	2.212	60.883	Severe
		s.d.	4.981	0.377	6.851	
		%CV	18.0%	17.1%	11.3%	
N-Lauroylsarcosine, sodium salt	10%	1	8.7	3.117	55.4	
		2	8.7	3.764	65.1	
		3	8.3	3.981	68.1	
		4	6.7	4.415	72.9	
		5	6.7	3.227	55.1	
		6	7.7	3.411	58.8	
		mean	7.800	3.653	62.567	Severe
		s.d.	0.927	0.496	7.282	
		%CV	11.9%	13.6%	11.6%	
Allyl alcohol	100%	1	116.7	1.552	139.9	
		2	112.3	1.953	141.6	
		3	90.3	1.856	118.2	
		4	97.7	1.578	121.3	
		5	80	1.945	109.2	
		6	67.3	2.804	109.4	
		mean	94.050	1.948	123.267	Severe
		s.d.	18.902	0.455	14.370	
		%CV	20.1%	23.4%	11.7%	
Butyrolactone	100%	1	35	0.38	40.7	
		2	33.3	0.506	40.9	
		3	31	0.291	35.4	
		4	31.7	0.747	42.9	
		5	39.7	0.721	50.5	
		6	34.7	0.326	39.6	

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		mean	34.233	0.495	41.667	Moderate
		s.d.	3.112	0.199	4.992	
		%CV	9.1%	40.2%	12.0%	
1-Phenyl-3-pyrazolidone	20%	1	11.5	0.156	13.8	
		2	11.5	0.093	12.9	
		3	9.8	0.11	11.5	
		4	11.2	0.114	12.9	
		5	9.8	0.145	12	
		6	12.5	0.237	16.1	
		mean	11.050	0.143	13.200	Mild
		s.d.	1.063	0.052	1.630	
		%CV	9.6%	36.4%	12.3%	
Methanol	100%	1	76.8	1.552	100.1	
		2	64.5	0.917	78.3	
		3	71.2	2.088	102.5	
		4	70.8	1.795	97.8	
		5	78.2	1.333	98.2	
		6	80.8	2.5	118.3	
		mean	73.717	1.698	99.200	Severe
		s.d.	5.993	0.560	12.777	
		%CV	8.1%	33.0%	12.9%	
Thiourea	20%	1	86	4.373	151.6	
		2	71	3.379	121.7	
		3	79	4.357	144.4	
		4	94	4.342	159.1	
		5	91	6.263	184.9	
		6	93.7	3.524	146.5	
		mean	85.783	4.373	151.367	Severe
		s.d.	9.187	1.028	20.672	
		%CV	10.7%	23.5%	13.7%	
Ethanol	100%	1	29.8	1.627	54.2	
		2	21.2	1.907	49.8	
		3	21.5	1.686	46.8	
		4	23.8	1.394	44.7	
		5	19.5	1.011	34.7	
		6	18.2	1.73	44.1	
		mean	22.333	1.559	45.717	Moderate
		s.d.	4.123	0.316	6.555	
		%CV	18.5%	20.3%	14.3%	
Ethyl acetoacetate	100%	1	28.3	0.215	31.6	
		2	25.3	0.043	26	
		3	20.7	0.06	21.6	
		4	23.3	0.307	27.9	
		5	25.3	0.023	25.7	
		6	21	0.049	21.7	
		mean	23.983	0.116	25.750	Moderate
		s.d.	2.907	0.117	3.809	
		%CV	12.1%	100.3%	14.8%	
Pyridine	100%	1	47.5	5.145	124.7	
		2	43.8	3.653	98.6	
		3	40.8	3.044	86.5	
		4	42.2	3.207	90.3	
		5	42.8	4.309	107.5	
		6	49.5	4.733	120.5	
		mean	44.433	4.015	104.683	Severe
		s.d.	3.357	0.849	15.705	
		%CV	7.6%	21.1%	15.0%	
Dimethyl sulfoxide	100%	1	6.7	0.218	9.9	
		2	5.3	0.159	7.7	
		3	8.7	0.196	11.6	
		4	4	0.291	8.4	
		5	5.7	0.23	9.1	
		6	7.7	0.134	9.7	
		mean	6.350	0.205	9.400	Mild
		s.d.	1.704	0.056	1.354	
		%CV	26.8%	27.1%	14.4%	

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2-Methoxyethanol	100%	1	48.7	0.879	61.8	
		2	39.7	0.685	49.9	
		3	52.7	0.751	63.9	
		4	37.7	0.732	48.6	
		5	52.7	1.044	68.3	
		6	39	0.713	49.7	
		mean	45.083	0.801	57.033	Severe
		s.d.	7.066	0.137	8.632	
%CV	15.7%	17.1%	15.1%			
Methylisobutyl ketone	100%	1	11.5	0.504	19.1	
		2	14.5	0.395	20.4	
		3	8.5	0.359	13.9	
		4	7.5	0.94	21.6	
		5	13.5	0.335	18.5	
		6	11.5	0.74	22.6	
		mean	11.167	0.546	19.350	Mild
		s.d.	2.733	0.244	3.073	
%CV	24.5%	44.7%	15.9%			
Dibenzoyl-L-tartaric acid	20%	1	90	0.523	97.9	
		2	81	0.425	87.4	
		3	53.7	0.453	60.5	
		4	63.7	0.485	70.9	
		5	87.7	0.194	90.6	
		6	75.3	0.416	81.6	
		mean	75.233	0.416	81.483	Severe
		s.d.	14.166	0.116	13.711	
%CV	18.8%	27.8%	16.8%			
Imidazole	20%	1	41.7	2.096	73.1	
		2	32.7	1.416	53.9	
		3	27.7	1.412	48.9	
		4	52	1.386	72.8	
		5	36.7	1.68	61.9	
		6	51.3	1.597	75.3	
		mean	40.350	1.598	64.317	Severe
		s.d.	9.893	0.271	11.151	
%CV	24.5%	17.0%	17.3%			
2-Aminophenol	20%	1	9.2	0.093	10.6	
		2	10.8	0.056	11.7	
		3	12.2	0.065	13.1	
		4	10.8	0.039	11.4	
		5	12.8	0.083	14.1	
		6	9.5	0.526	17.4	
		mean	10.883	0.144	13.050	Mild
		s.d.	1.426	0.188	2.473	
%CV	13.1%	131.1%	18.9%			
1,2,4-Trimethylbenzene	100%	1	12.2	0.34	17.3	
		2	13.8	0.532	21.8	
		3	12.5	0.383	18.3	
		4	12.8	0.786	24.6	
		5	9.8	1.216	28.1	
		6	13.8	0.212	17	
		mean	12.483	0.578	21.183	Mild
		s.d.	1.473	0.369	4.490	
%CV	11.8%	63.8%	21.2%			
1,2,3-Trichloropropane	100%	1	6	6.151	98.3	
		2	11.3	4.191	74.2	
		3	6.3	4.491	73.7	
		4	7	7.751	123.3	
		5	8.3	4.511	76	
		6	7.3	6.271	101.4	
		mean	7.700	5.561	91.150	Severe
		s.d.	1.940	1.398	20.056	
%CV	25.2%	25.1%	22.0%			
Aluminum hydroxide	20%	1	8.7	0.012	8.9	
		2	9.7	0.004	9.7	
		3	10	0.015	10.2	

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		4	13	0.011	13.2	
		5	6	0.022	6.3	
		6	11	0.006	11.1	
		mean	9.733	0.012	9.900	Mild
		s.d.	2.339	0.006	2.299	
		%CV	24.0%	55.5%	23.2%	
Diacetone alcohol	100%	1	32.5	4.507	100.1	
		2	31.2	4.119	92.9	
		3	34.8	6.509	132.5	
		4	25.5	3.775	82.1	
		5	32.8	3.064	78.8	
		6	29.8	2.74	70.9	
		mean	31.100	4.119	92.883	Severe
		s.d.	3.212	1.341	21.997	
		%CV	10.3%	32.6%	23.7%	
Laurylsulfobetaine	10%	1	14.3	6.259	108.2	
		2	19.3	4.404	85.4	
		3	19	7.4	130	
		4	10	3.743	66.2	
		5	13.3	5.543	96.5	
		6	21.3	7.102	127.9	
		mean	16.200	5.742	102.367	Severe
		s.d.	4.334	1.462	24.819	
		%CV	26.8%	25.5%	24.2%	
2,4-Dichloro-5-sulfamoylbenzoic acid	20%	1	24	-0.011	23.8	
		2	21	-0.014	20.8	
		3	15.3	-0.011	15.2	
		4	11.7	-0.003	11.6	
		5	22	-0.012	21.8	
		6	22	-0.008	21.9	
		mean	19.333	-0.010	19.183	Mild
		s.d.	4.761	0.004	4.723	
		%CV	24.6%	-39.3%	24.6%	
Propyl-4-hydroxybenzoate	20%	1	8	0.008	8.1	
		2	4	0.169	6.5	
		3	6.3	0.063	7.3	
		4	4	0.014	4.2	
		5	4.7	0.089	6	
		6	4	0.053	4.8	
		mean	5.167	0.066	6.150	Mild
		s.d.	1.650	0.059	1.476	
		%CV	31.9%	89.3%	24.0%	
3-Glycidoxypropyltrimethoxysilane	100%	1	19.5	0.213	22.7	
		2	21.2	0.015	21.4	
		3	20.5	0.005	20.6	
		4	12.5	0.111	14.2	
		5	10.5	0.023	10.8	
		6	15.5	0.025	15.9	
		mean	16.617	0.065	17.600	Mild
		s.d.	4.472	0.082	4.693	
		%CV	26.9%	125.3%	26.7%	
Triethanolamine	100%	1	3.2	0.029	3.6	
		2	3.2	0.03	3.6	
		3	1.8	0.016	2.1	
		4	3.5	0.043	4.1	
		5	2.8	0.018	3.1	
		6	1.2	0.016	1.4	
		mean	2.617	0.025	2.983	Mild
		s.d.	0.913	0.011	1.030	
		%CV	34.9%	42.3%	34.5%	
Phenylbutazone	20%	1	0.3	-0.005	0.3	
		2	0.3	-0.012	0.1	
		3	0.3	-0.015	0.1	
		4	1	-0.007	0.9	
		5	0.7	0.005	0.7	
		6	1.3	-0.013	1.1	

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		mean	0.650	-0.008	0.533	Mild
		s.d.	0.428	0.007	0.427	
		%CV	65.8%	-93.6%	80.1%	
Sodium oxalate	20%	1	0.3	0.092	1.7	
		2	1	0.028	1.4	
		3	2.3	0.108	3.9	
		4	1.7	0.128	3.6	
		5	2	0.15	4.2	
		6	2.7	0.115	4.4	
		mean	1.667	0.104	3.200	Mild
		s.d.	0.882	0.042	1.310	
		%CV	52.9%	40.4%	40.9%	
Tetraaminopyrimidine sulfate	20%	1	2.7	-0.009	2.5	
		2	3	0.002	3	
		3	3	-0.009	2.9	
		4	1	0.007	1.1	
		5	1	-0.007	0.9	
		6	4.7	-0.004	4.6	
		mean	2.567	-0.003	2.500	Mild
		s.d.	1.404	0.007	1.367	
		%CV	54.7%	-196.0%	54.7%	
Magnesium carbonate	20%	1	1	0.02	1.3	
		2	0	0.015	0.2	
		3	0	0.023	0.4	
		4	1	0.012	1.2	
		5	0	0.015	0.2	
		6	1	0.014	1.2	
		mean	0.500	0.017	0.750	Mild
		s.d.	0.548	0.004	0.536	
		%CV	109.5%	25.1%	71.4%	
Betaine monohydrate	20%	1	6.7	0.015	6.9	
		2	1	0.026	1.4	
		3	1	0.027	1.4	
		4	5	0.02	5.3	
		5	1.7	0.055	2.5	
		6	3	0.029	3.4	
		mean	3.067	0.029	3.483	Mild
		s.d.	2.339	0.014	2.219	
		%CV	76.3%	48.5%	63.7%	
Triton X-155	10%	1	2.7	0.003	2.7	
		2	3.7	-0.006	3.6	
		3	4.7	0	4.7	
		4	0.7	0.004	0.7	
		5	1.7	0.02	2	
		6	4.7	0.032	5.1	
		mean	3.033	0.009	3.133	Mild
		s.d.	1.633	0.014	1.669	
		%CV	53.8%	161.4%	53.3%	
EDTA, di-potassium salt	20%	1	0.3	0.024	0.7	
		2	1	0.02	1.3	
		3	0.3	-0.004	0.3	
		4	0.3	-0.004	0.3	
		5	1	0.024	1.4	
		6	1.7	0	1.7	
		mean	0.767	0.010	0.950	Mild
		s.d.	0.572	0.014	0.599	
		%CV	74.5%	140.3%	63.1%	
BRIJ-35	10%	1	1.7	0.012	1.8	
		2	0.7	0.001	0.7	
		3	0.7	0	0.7	
		4	2	-0.006	1.9	
		5	0.7	-0.008	0.6	
		6	0.7	-0.009	0.5	
		mean	1.083	-0.002	1.033	Mild
		s.d.	0.601	0.008	0.638	
		%CV	55.5%	-471.9%	61.7%	

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Data from Dr. Freddy Van Goethem**

Substance	Concentration	Cornea number	Opacity	Permeability	<i>In Vitro</i> Score ¹	<i>In Vitro</i> Classification
Petroleum ether	100%	1	3.2	0.006	3.3	
		2	0.5	0.02	0.8	
		3	3.8	0.035	4.4	
		4	2.2	0.008	2.3	
		5	2.8	0.006	2.9	
		6	-1.2	0.012	-1	
		mean	1.883	0.015	2.117	Mild
s.d.	1.885	0.011	1.934			
%CV	100.1%	78.2%	91.4%			
Anthracene	20%	1	3	0.001	3	
		2	0.3	-0.009	0.2	
		3	1.7	0.005	1.7	
		4	0.3	0.004	0.4	
		5	2.7	0.007	2.8	
		6	0.3	0.01	0.5	
		mean	1.383	0.003	1.433	Mild
s.d.	1.262	0.007	1.253			
%CV	91.3%	220.1%	87.4%			
Dimethylbiguanide	20%	1	0	0.016	0.2	
		2	2	0.014	2.2	
		3	1	0.036	1.5	
		4	-1.7	0.034	-1.2	
		5	-0.7	0.457	6.2	
		6	3.3	0.026	3.7	
		mean	0.650	0.097	2.100	Mild
s.d.	1.829	0.177	2.617			
%CV	281.5%	181.7%	124.6%			
MYRJ-45	10%	1	2.8	0.012	3	
		2	-1.2	0.001	-1.1	
		3	0.2	0.005	0.2	
		4	-0.5	0.002	-0.5	
		5	0.5	0.001	0.5	
		6	0.5	0.009	0.6	
		mean	0.383	0.005	0.450	Mild
s.d.	1.356	0.005	1.407			
%CV	353.6%	92.1%	312.6%			
Hexane	100%	1	0.7	0.002	0.7	
		2	4.3	0.002	4.4	
		3	2.3	0	2.3	
		4	-0.3	0.007	-0.2	
		5	-0.3	0.003	-0.3	
		6	1.3	0.002	1.4	
		mean	1.333	0.003	1.383	Mild
s.d.	1.759	0.002	1.775			
%CV	131.9%	87.7%	128.3%			
2-Mercaptopyrimidine	20%	1	0	-0.005	-0.1	
		2	0	-0.004	-0.1	
		3	0	-0.006	-0.1	
		4	0	0.001	0	
		5	0	-0.005	-0.1	
		6	-1	-0.004	-1.1	
		mean	-0.167	-0.004	-0.250	Mild
s.d.	0.408	0.002	0.418			
%CV	-244.9%	-64.8%	-167.3%			
Iminodibenzyl	20%	1	0	0.002	0	
		2	0	0.002	0	
		3	0	-0.002	0	
		4	0	-0.003	0	
		5	1	0.001	1	
		6	0	-0.004	-0.1	
		mean	0.167	-0.001	0.150	Mild
s.d.	0.408	0.003	0.418			
%CV	244.9%	-398.7%	278.9%			
DL-Glutamic acid	20%	1	0.3	-0.004	0.3	
		2	-0.7	0.003	-0.6	
		3	0.3	-0.012	0.2	

**Intralaboratory CV Analysis of BCOP -
Data from Dr. Freddy Van Goethem**

Substance	Concentration	Cornea number	Opacity	Permeability	<i>In Vitro</i> Score ¹	<i>In Vitro</i> Classification
		4	-0.7	-0.005	-0.7	
		5	0.3	-0.006	0.2	
		6	-0.7	-0.008	-0.8	
		mean	-0.200	-0.005	-0.233	Mild
		s.d.	0.548	0.005	0.516	
		%CV	-273.9%	-93.1%	-221.3%	

Abbreviations: CV = Coefficient of variation; SD = Standard deviation

¹*In Vitro* Score = Opacity + (15 x permeability value)