Appendix G4

Dataset Received from L'OREAL Advanced Research for an In-House Porcine Corneal Opacity and Permeability Assay

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Preliminary comments :

L'OREAL Advanced Research understood very early the usefulness of isolated cornea assay as a predictive tool for ocular irritancy. Due to constraints of supply and use of bovine eyes, we decided to use porcine corneas.
We have developped an in-house PCOP protocol to provide adequate safety data for cosmetic ingredients. Our PCOP protocol is basically that previously described by P. Gautheron, with some changes linked to species.

For liquid form and water-soluble materials we obtained a prediction model based on only one endpoint (O.D. $_{30}$) allowing to distinguish non irritating compounds (MAS ≤ 15 if O.D. $_{30}$ < 0. 35) from irritating compounds (MAS > 15 if O.D. $_{30}$ ≥ 0. 35).

Using this two classes scheme, 49 of the 50 raw ingredients tested in our PCOP assay were accurately classified. Only one was over-predicted. Agreement between in vivo and *in vitro* classification was high (concordance 98% -Kappa = 0. 96, p < 0.01).

	Observed class (*in vivo*)	
Predicted class (*in vitro*)	Mild irritant MAS ≤15	Moderate/ Irritant / Severe MAS >15
Mild irritant MAS ≤15	25	0
Moderate/ Irritant / Severe MAS >15	1	24

As MAS were available for 43 of the materials, a linear regression was carried out to predict the Draize Maximum Average Score (MAS).
An equation PM was obtained to predict MAS value, but despite satisfactory statistical coefficients - R^2Y= 0.84, concordance = 90 % - this algorithm is not recommended .Data analysis showed that 95% confidence interval was wide and there was too much uncertainty of the MAS prediction for it to be used.

We used the Draize test classification scheme according to French regulations applied to cosmetics (J. O. R. F. June 1992.) as follows :

MAS value	Class
≤ 15	mild irritant
>15 and ≤ 30	moderate irritant
> 30 and ≤ 50	irritant
> 50	severe irritant

Table 1 summarized our PCOP results obtained on 50 liquid and water-soluble compounds, selected on the basis of preexisting *in vivo* data - including 32 surfactants, 7 polymers, 5 solvents, 4 active ingredients and 2 dyes.

All compounds were tested *in vitro* at the same concentration as *in vivo*.

Some of them , including references , were tested in both PCOP and BCOP - See Table II and III for details .

Taking into account those results PCOP seems to be more suitable to predict class irritation than BCOP. Future work need to be done to complete this comparison .

Abbreviations used :
PCOP = Porcine cornea permeability and opacity assay;
BCOP = Bovine cornea permeability and opacity assay;
MAS = maximum average score;
OP_{10} or OP_{30} = opacity induced by a 10-min or 30 min- exposure of corneas, respectively;
$O.D._{10}$ or $O.D._{30}$ = optical density measured after a 10-min or 30 min- exposure of corneas, respectively;
PM = prediction model.

Table 1: PCOP results obtained on 50 liquid and water-soluble compounds

		PCOP Data				Predicted data		In vivo data (historical)	
N° product	Concentration tested	OP_{10}	$O.D._{10}$	OP_{30}	$O.D._{30}$	PCOP class	MAS (Class deducted)	MAS	class
1	0.5%	4	-0.043	0.3	0.084	Mild	10.2 (Mild)	0.7	Mild
2	10%	-0.3	-0.006	6.3	0.008	Mild	8.3 (Mild)	2.3	Mild
3	20%	-1	-0.003	0.4	0.003	Mild	8.2 (Mild)	3.7	Mild
4	10%	6.7	0.041	53.9	0.092	Mild	10.4 (Mild)	4.0	Mild
5	5%	7.7	0.042	12.7	0.028	Mild	8.8 (Mild)	4.7	Mild
6	10%	-0.7	-0.001	2.7	0.096	Mild	10.5 (Mild)	5.3	Mild
7	10%	-1.3	-0.004	5.7	0.044	Mild	9.2 (Mild)	5.7	Mild
8	100%	1.7	0.014	1.3	0.066	Mild	9.8 (Mild)	8.5	Mild
9	100%	2.6	0.036	-2.7	-0.034	Mild	7.2 (Mild)	9.8	Mild
10	3.1%	0.3	-0.009	-0.3	-0.008	Mild	7.9 (Mild)	10.7	Mild
11	100%	-5.6	-0.002	-4.0	0.003	Mild	8.2 (Mild)	10.7	Mild
12	100%	3.3	-0.002	2.0	0.013	Mild	8.4 (Mild)	10.8	Mild
13	100%	4.3	-0.002	39.0	0.133	Mild	11.5 (Mild)	11.0	Mild
14	100%	5	-0.004	-2.3	-0.003	Mild	8.0 (Mild)	11.3	Mild
15	10%	0.3	-0.017	2.8	0.021	Mild	8.6 Mild)	11.3	Mild
16	5%	5.6	0.421	19.0	1.040	**Irrg.**	29.4 (Mod.)	12.0	Mild
17	100%	1	0.007	4.7	0.028	Mild	8.8 (Mild)	12.0	Mild
18	100%	3	-0.001	12.3	-0.007	Mild	7.9 (Mild)	12.3	Mild
19	6.7%	4.7	0.212	15.0	0.296	Mild	15.3 (Mod.)	12.7	Mild
20	100%	7.3	0.056	21.2	0.014	Mild	8.4 (Mild)	12.8	Mild

		PCOP Data				Predicted data		In vivo data (historical)	
N° product	Concentration tested	OP_{10}	$O.D._{10}$	OP_{30}	$O.D._{30}$	PCOP class	MAS (Class deducted)	MAS	class
21	2%	3.3	0.042	5.0	0.056	Mild	9.5 (Mild)	13.0	Mild
22	100%	9.3	0.049	17.0	0.089	Mild	Not AppL	NA	Mild
23	100%	2	0.053	7.3	-0.007	Mild	Not AppL	NA	Mild
24	100%	-2.4	0.012	-1.0	0.003	Mild	Not AppL	NA	Mild
25	10%	0.3	0.003	2.7	0.014	Mild	Not AppL	NA	Mild
26	100%	20.7	0.118	67.1	0.367	Irrg.	16.9 (Mod.)	16.0	Mod.
27	6%	16	1.641	14.0	2.060	Irrg.	38.7 (Irr. to Sev.)	30.6	Irr.
28	10%	6.3	0.516	40.5	2.081	Irrg.	38.8 (Irr. to Sev.)	31.0	Irr.
29	0.5%	23	0.739	25.5	0.451	Irrg.	18.8 (mod.)	31.0	Irr.
30	10%	31.3	0.876	38.7	1.268	Irrg.	32.5 (Irr. to Sev.)	31.3	Irr.
31	10%	7.3	0.343	7.7	2.202	Irrg.	39.2 (Irr. to Sev.)	31.7	Irr.
32	10%	24.7	0.979	17.3	0.918	Irrg.	27.8 (Irr. to Sev.)	31.7	Irr.
33	10%	13.7	1.041	3.0	1.764	Irrg.	37.2 (Irr. to Sev.)	32.7	Irr.
34	10%	20	0.868	46.0	1.488	Irrg.	34.9 (Irr. to Sev.)	33.7	Irr.
35	6%	14	0.659	32.5	1.896	Irrg.	37 (Irr. to Sev.)	34.7	Irr.
36	6%	35.3	0.919	30.3	1.548	Irrg.	35.5 (Irr. to Sev.)	35.3	Irr.
37	10%	13	0.613	11.7	1.123	Irrg.	30.6 (Mod.)	35.7	Irr.
38	10%	16.7	1.917	22.3	2.132	Irrg.	38.9 (Irr. to Sev.)	37.4	Irr.
39	10%	14.3	0.750	16.7	2.016	Irrg.	38.6 (Irr. to Sev)	39.3	Irr.
40	10%	9.3	2.433	22.3	1.738	Irrg.	37 (Irr. to Sev.)	40.3	Irr.
41	10%	8.3	0.668	23.0	1.667	Irrg.	36.5 (Irr. to Sev.)	40.7	Irr.
42	10%	1.3	0.308	38.3	1.714	Irrg.	36.8 (Mod.)	43.0	Irr.
43	10%	11.3	0.786	18.0	2.094	Irrg.	38.9 (Irr. to Sev.)	45.0	Irr.
44	10%	29	1.568	33.3	1.978	Irrg.	38.4	46.0	Irr.

		PCOP Data				Predicted data		In vivo data (historical)	
N° product	Concentration tested	OP_{10}	O.D.$_{10}$	OP_{30}	O.D.$_{30}$	PCOP class	MAS (Class deducted)	MAS	class
							(Irr. to Sev.)		
45	10%	23	1.288	19.7	2.174	Irrg.	39.1 (Irr. to Sev.)	47.2	Irr.
46	10%	17.3	0.995	8.0	1.538	Irrg.	Not AppL	63.7	Sev.
47	10%	21.3	1.618	11.3	1.679	Irrg.	Not AppL	63.7	Sev
48	10%	16.7	0.495	27.3	0.878	Irrg.	Not AppL	NA	Irr.
49	10%	6.7	0.232	18.0	1.112	Irrg.	Not AppL	NA	Irr.
50	10%	10.7	0.639	48.3	0.997	Irrg.	Not AppL	NA	Irr.

OP_{10}. OP_{30}. O.D. $_{10}$ O.D. $_{30}$: Each data represents mean corrected value of three corneas.
NA: not available.
Not AppL: Not applicable

Predicted class is determined by the PM: O.D. $_{30} < 0.35 \Rightarrow$ predict nonirritating (MAS ≤15 - mild irritant) - O.D. $_{30} \geq$
$0.35 \Rightarrow$ predict irritating (Irrg.) corresponding to MAS >15, covering three French Draize classes - moderate, irritant, severe.
Predicted MAS is calculated using this algorithm = 8. 08 + 26. 16 x O. D. $_{30} -$ 5. 47 x O.D $_{30}^2$, with deduction of Draize class in brackets. Evaluation of this PM was based on three classes – Mild irritant (MAS ≤ 15), Moderate irritant
(15< MAS ≤ 30) , irritant to severe (MAS >30). Data analysis showed that 95% confidence interval was wide. There is too much uncertainty of the MAS prediction for it to be used.

Table II - References tested in PCOP and BCOP with historical data (N = 8)

		In vivo data (historical)		PCOP data					BCOP data						
N° PRODUCT	CONC	MAS	In vivo Class	OP_{10}	$O.D._{10}$	OP_{30}	$O.D._{30}$	Predicted class	OP_{10}	$O.D._{10}$	Score 10	OP_{30}	$O.D._{30}$	Score 30	Predicted Class
Propylene glycol (n°20)	100%	12.8	Mild	7.3	0.056	21.2	0.014	Mild	NA	NA	NA	11,7	0.001	1.8	1
Sodium dodecyl sulfate (n°38)	10%	37.4	Irr.	16.7	1.917	22.3	2.132	Irrg	NA	NA	NA	1.5	0.424	18	2
Triton X100 (n°40)	10%	40.3	Irr.	9.3	2.433	22.3	1.738	Irrg	3.2	2.717	43.9	2.1	5.197	79.5	3
Vaseline oil	100%	NI	Mild	-0.3	0.008	-1	-0.03	Mild	NA	NA	NA	NA	0.005	4.0	1
Tween 20 (n° 7)	10%	5.7	Mild	-1.3	0.004	5.7	0.044	Mild	0.3	0.003	0.3	1.8	0.001	1.8	1
Ethanol	100%	37 (at 1h)	Irr.	40	1.260	58.2	0.676	Irrg	26.9	2.912	70.6	NA	NA	NA	3
Ethanol	50%	NA	NA	8.3	0.036	18	0.075	Mild	NA	NA	22.9	NA	1.117	31.3	3
Ethanol	10%	NA	NA	-2.3	0.014	0.3	-0.016	Mild	NA	NA	NA	NA	0.021	0.5	1
Lactic Acid	10%	31.2	Irr.	78	0.928	271	1.552	Irrg	15.2	0.029	15.6	75.2	0.439	81.7	3
Hexadecyl trimethyl ammonium bromide (CTAB)	0.5%	NA	NA	42.3	0.562	53.0	1.270	Irrg	NA	NA	43.3	48.2	5.112	124.9	3

Numbers in brackets refer to table I.

NA: not available.

TABLE III: Results obtained on compounds tested in both PCOP and BCOP (N = 15)

		In vivo data (historical)		PCOP data					BCOP data						
N°	CONC TESTED	MAS	Classe	OP_{10}	$O.D._{10}$	OP_{30}	$O.D._{30}$	Predicted Class	OP_{10}	$O.D._{10}$	Score 10	OP_{30}	$O.D._{30}$	Score 30	Predicted Class
8	100%	8,5	Mild	1,7	0,014	1,3	0,066	Mild	NA	NA	NA	0,8	-0,006	0,7	1
13	100%	11,0	Mild	4,3	-0,002	39,0	0,133	Mild	4,4	0,040	5,0	24,7	0,958	39,1	2 or 3
18	100%	12,3	Mild	3,0	-0,001	12,3	-0,007	Mild	NA	NA	NA	5,8	0,057	6,6	1
20	100%	12,8	Mild	7,3	0,056	21,2	0,014	Mild	NA	NA	NA	11,7	0,424	18,0	2
26	100%	16,0	Mod.	20,7	0,118	67,1	0,367	Irrg	71,5	0,971	86,1	58,6	2,346	93,8	3
A	10%	30,3	Irrg	47,3	1,256	186,0	0,796	Irrg	12,1	1,505	34,7	51,3	4,107	112,9	3
30	10%	31,3	Irrg	31,3	0,876	38,7	1,268	Irrg	56,8	1,786	83,6	98,8	2,327	133,7	3
31	10%	31,7	Irrg	7,3	0,343	7,7	2,202	Irrg	2,5	1,058	18,4	3,0	3,238	51,6	2 or 3
32	10%	31,7	Irrg	24,7	0,979	17,3	0,918	Irrg	3,7	1,977	33,4	3,5	5,231	82,0	3
33	10%	32,7	Irrg	13,7	1,041	3,0	1,764	Irrg	0,3	0,860	13,2	2,7	4,439	69,8	3
39	10%	39,3	Irrg	14,3	0,750	16,7	2,016	Irrg	0,4	0,721	11,2	3,9	1,043	16,9	2
42	10%	43.0	Irrg	1,3	0,308	38,3	1,714	Irrg	5,7	1,082	22,0	6,1	5,392	86,9	3
43	10%	45,0	Irrg	11,3	0,786	18,0	2,094	Irrg	3,6	1,077	19,8	3,6	3,921	62,4	3
45	10%	47,2	Irrg	23,0	1,288	19,7	2,174	Irrg	5,1	3,098	51,6	2,3	5,040	77,9	3
46	10%	63,7	Irrg	17,3	0,995	8,0	1,538	Irrg	4,9	1,812	32,1	2,4	3,854	60,2	3

Product numbers refer to table I .

Our BCOP protocol is a variation on the original protocol developed by P. GAUTHERON .

Corneal score = OP + (15 x O.D.). Classification depends on corneal scores at 30 minutes completed with results at 10 minutes :

Score at 30 min	Class
≤ 10	Class 1 : mild irritant
10 - 25	Class 2 : moderate irritant if score 10 mn ≤ 10 Class 2 or 3 if 10 < score 10 mn ≤ 25 Class 3 : irritant to strong irritant if score 10 mn >25
25 - 55	Class 2 or 3 if score 10 mn ≤ 10 Class 3 : irritant to strong irritant if score 10 mn >10
>55	Class 3 : irritant to strong irritant