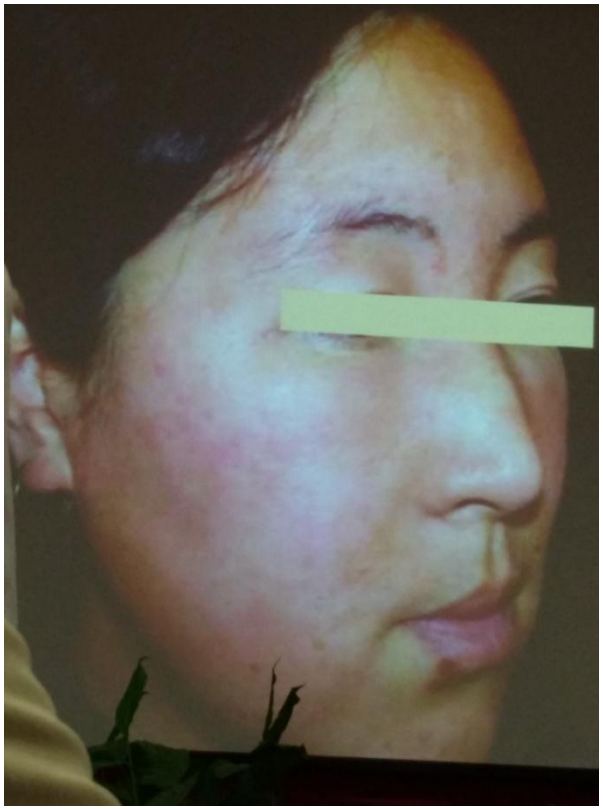
DPRA
（Direct Peptide Reaction Assay)

●皮肤致敏

- 引起变态性接触性皮炎（ACD）
- 评价化妆品原料安全性的一个很重要的标准要求



- 化妆品接触性皮炎占化妆品皮肤病的70％~90％
- 变态性接触性皮炎占20％

●皮肤致敏机制

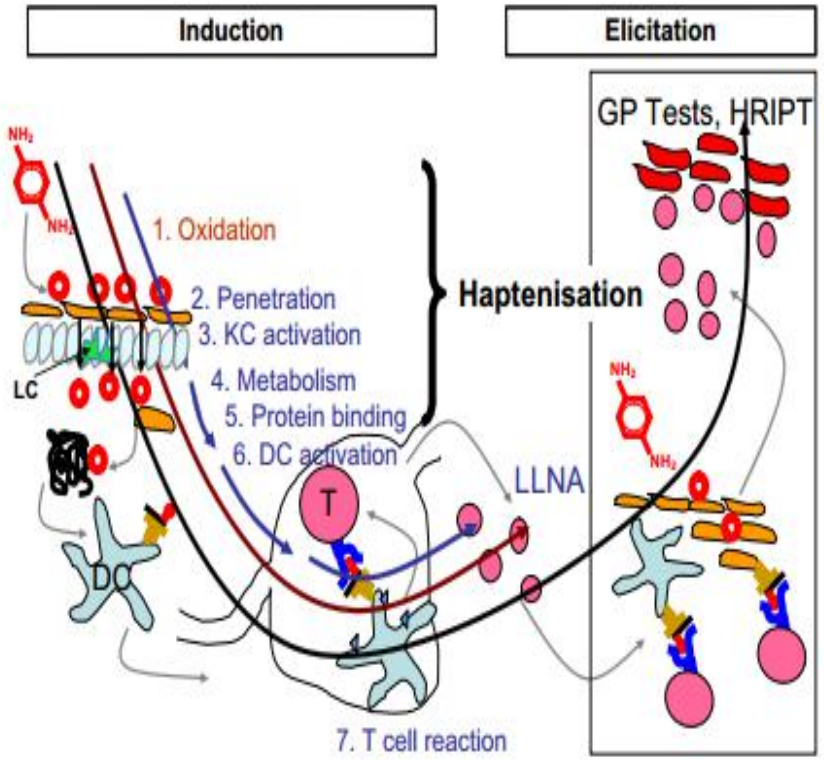


Fig. 1. Key events in skin sensitisation. Schematic representation of processes involved in the induction and elicitation phases of skin sensitisation by allergenic chemicals. KC: keratinocyte, DC: dendritic cell, StrC: stratum corneum, T: T cell, LLNA: local lymphnode assay, GP: guinea pig, HRIPT: human repeat insult patch test.

●皮肤致敏机制

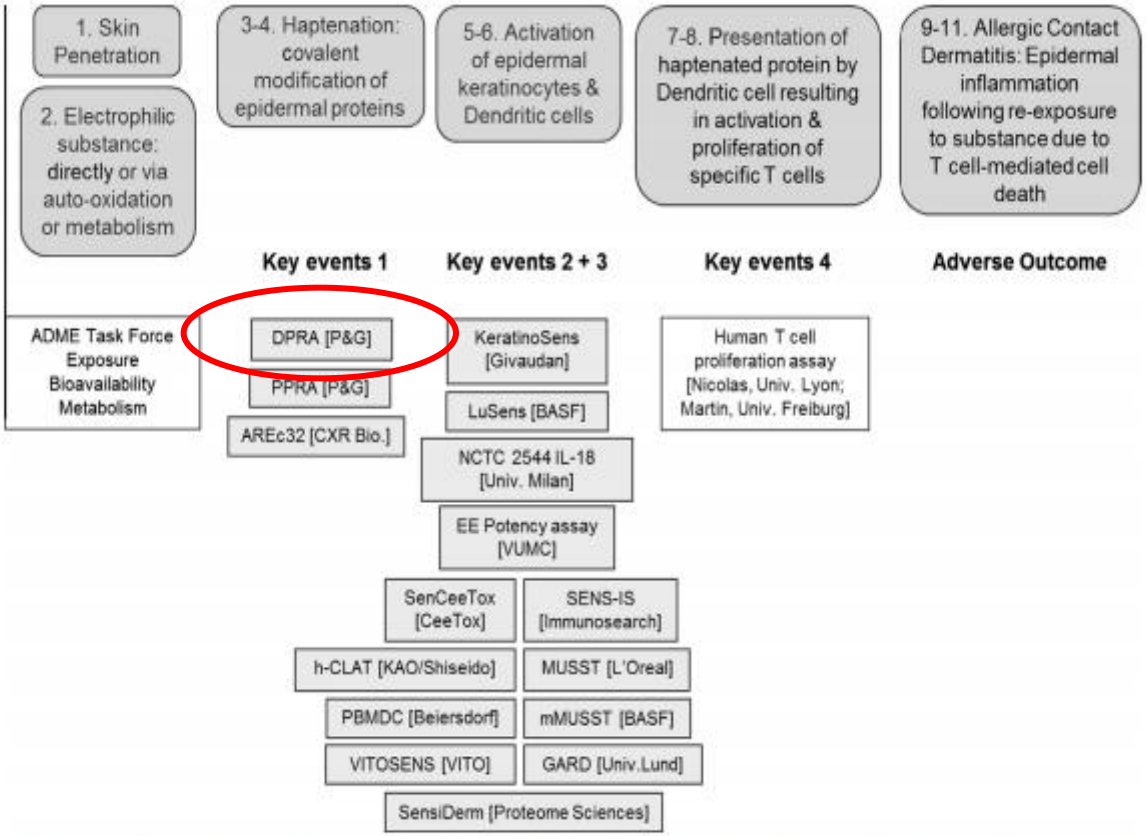


Fig. 1. Non-animal test methods and their alignment to the skin sensitisation AOP. Test methods analysed during of phase I of the Cosmetics Europe method evaluation study (grey boxes). Methods presented in white boxes represent Cosmetics Europe-funded studies to cover the steps in the AOP, which are currently not represented by a non-animal assay.

●DPRA实验原理

- 致敏物一般为小分子亲电物质
- 易与氨基酸结合，主要与半胱氨酸和赖氨酸

一般认为，如果某物质能与蛋白直接或者经过转化后结合，那么该物质具有致敏风险

- 设计了两条寡肽链（7个氨基酸），分别含有半胱氨酸和赖氨酸
- 当与受试物发生反应时，HPLC结果将显示寡肽链的峰面积变化

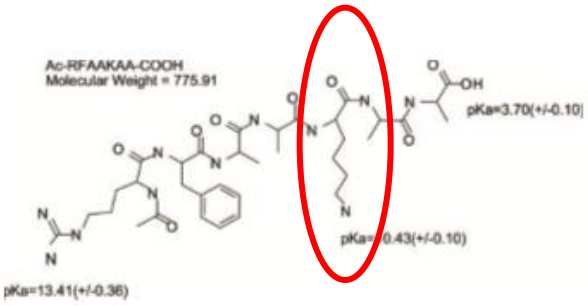


FIG. 1. Structure of synthetic peptide, Ac-RFAAKAA-COOH, showing the lysine side chain. The pKa of the amines is shown. The other synthetic peptides were similarly structured, except a cysteine or histidine residue was substituted for the lysine.

Ac-RFAACAA-COOH

Ac-RFAAKAA-COOH

●DPRA实验方法

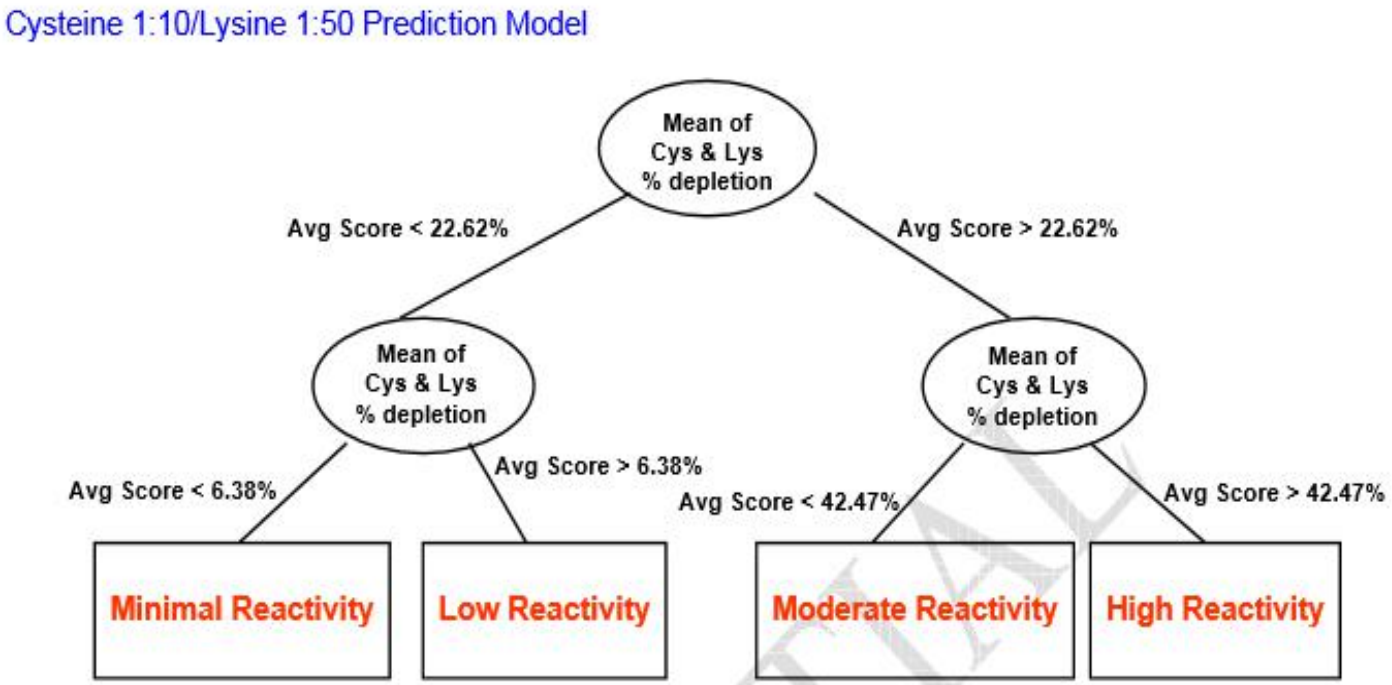
- 受试物测试母液浓度为100 mM
- 与Cysteine摩尔比为1:50；与Lysine摩尔比为1:10孵育24 h，用HPLC分别检测Cysteine和Lysine的峰面积变化

消除率计算方式，分别计算Cysteine和Lysine的消除率

$$\text{Percent Peptide Depletion} = \left[1 - \left(\frac{\text{Peptide Peak Area in Replicate Injection}}{\text{Mean Peptide Peak Area in Reference Controls } C}\right)\right] \times 100$$

通过计算两肽链平均消除率进行判断

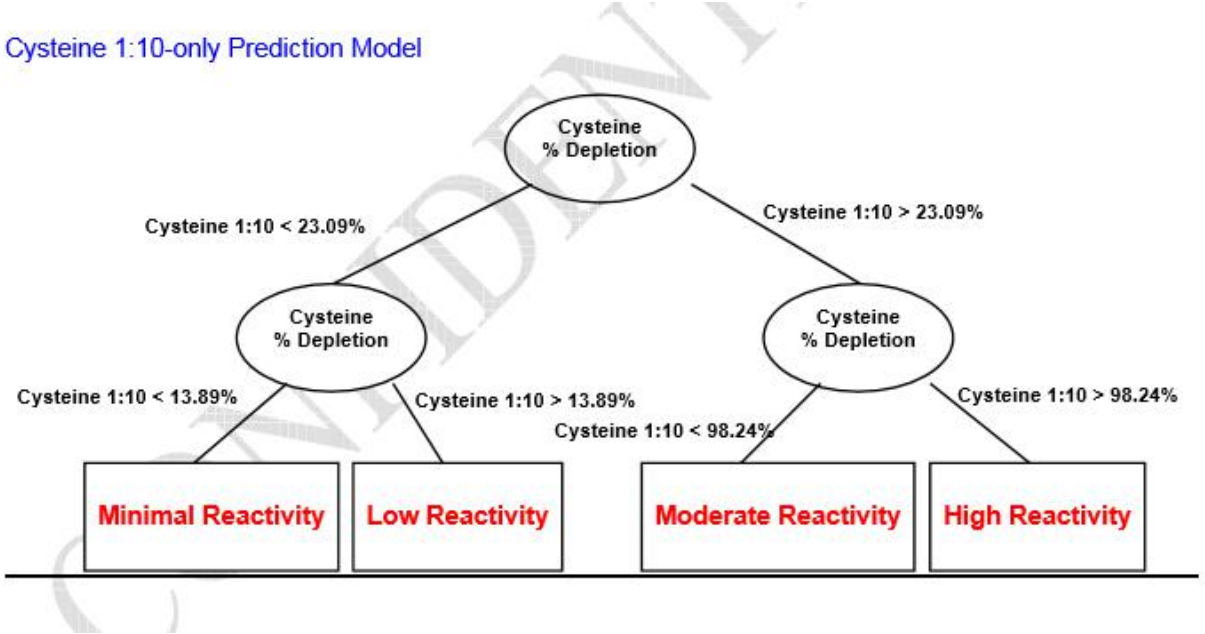
●DPRA实验结果判定



两肽链都未出现Co-elution的情况

●DPRA实验结果判定

- 若受试物与Lysine发生了Co-elution



- 若受试物与Cysteine发生了Co-elution，则无法判定

◆DPRA

- 宝洁（2004年已发表DPRA的使用数据）
- 2012年被OECD认证，收录于OECD 442C

宝洁进行了133个样品的测试，并与LLNA结果做比对

Table 33. Performance of the DPRA as evaluated from the results submitted to ECVAM by P&G

		Predicted Classification (based on classification tree model)		
		Non-Sensitiser	Sensitiser	total
Chemical Classification (based on LLNA)	Non-Sensitiser	30	6	36
	Sensitiser	13	84	97
	Total	43	90	133

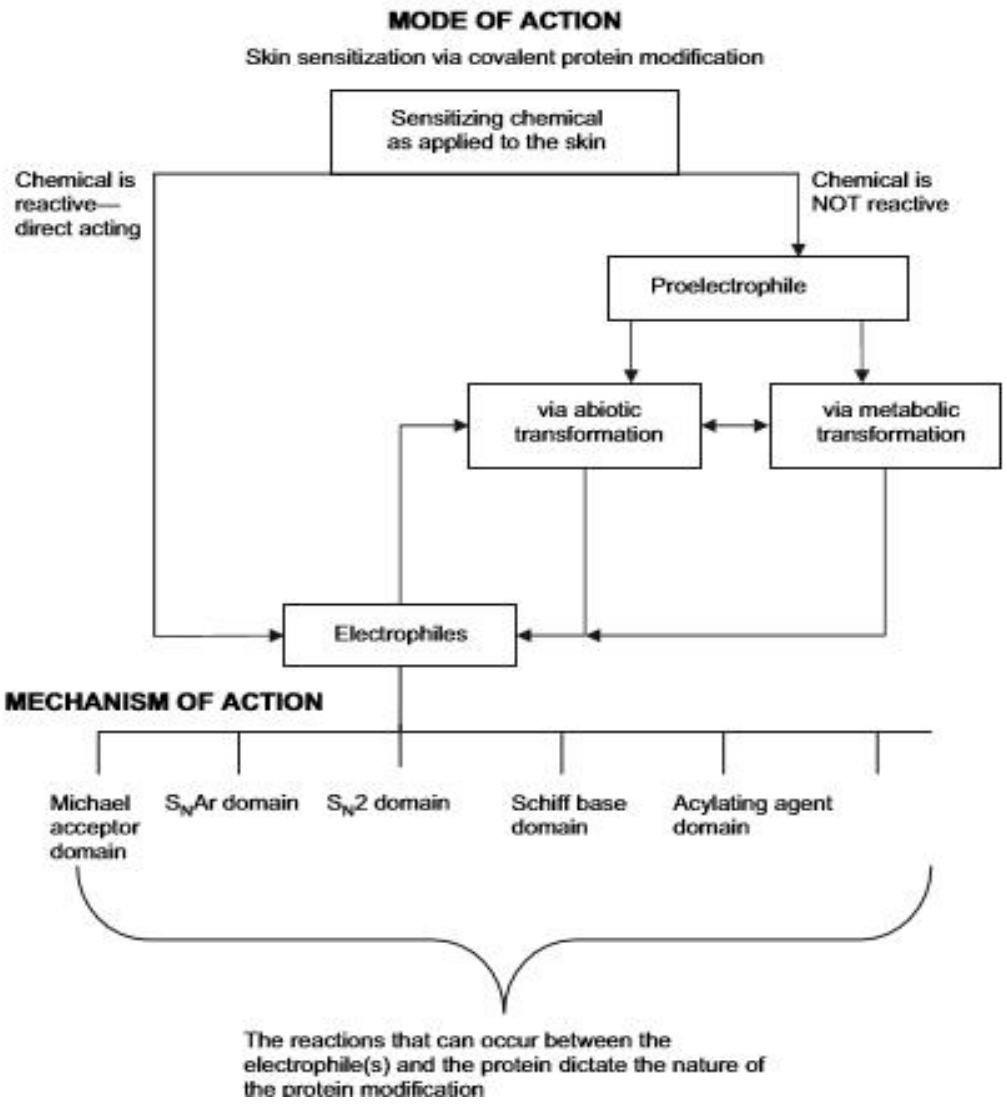
table statistics for the shadowed 2 x 2 table

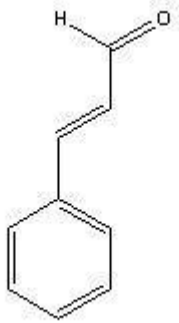
sensitivity:	87%
specificity:	83%
positive predictivity:	93%
negative predictivity:	70%
accuracy:	**86%**

DPRA，KeratinoSens，h-Clat，U-Sens比较可知，DPRA的预测能力最好

Bauch et al., Tox in Vitro 2011

◆DPRA：发生消除反应的结构特点





肉桂醛

●DPRA的不足

- 不能检测未知分子量的物质
- 不能做为单一实验使用
- 化学反应，无代谢功能
- 无法预测致敏潜力

●组合实验

Bauch et al., Tox in Vitro 2011

Sensitizer

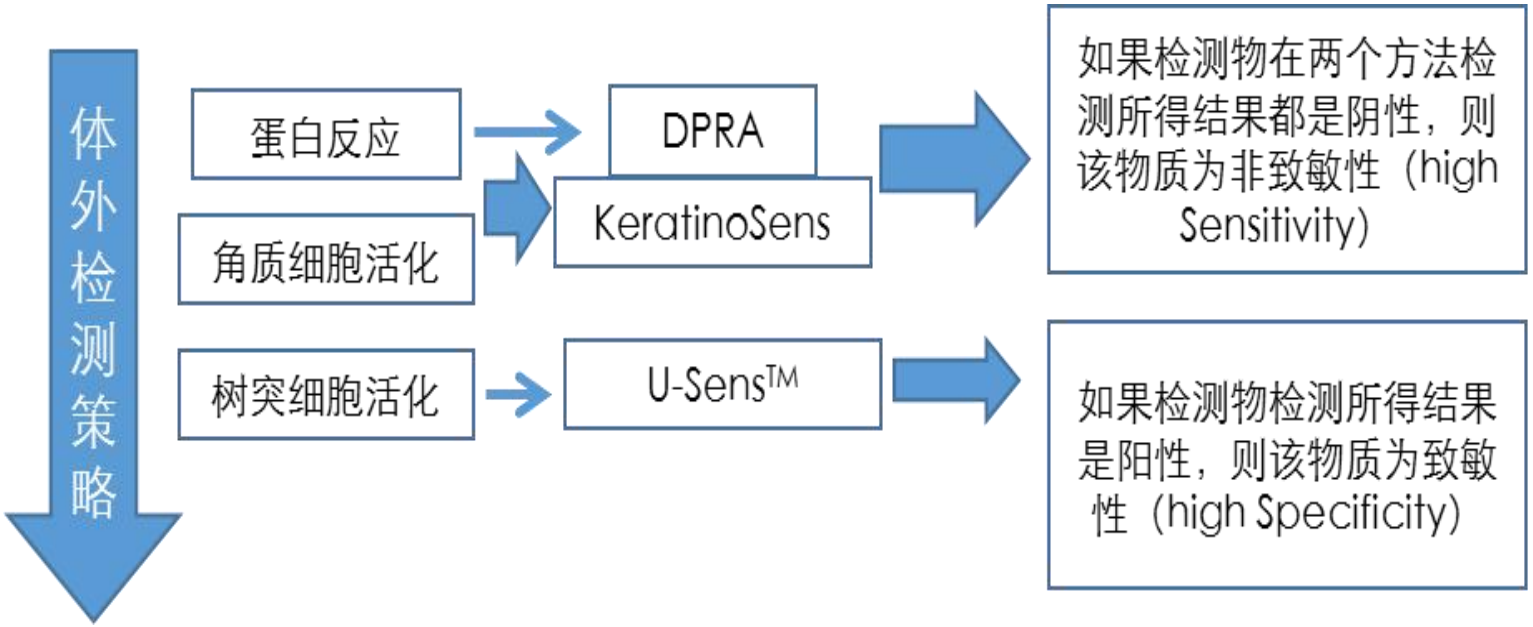
Non-Sensitizer

Substance	Human	LLNA	DPRA	KeratinoSens	Lusens	mMusst	h-CLAT
1-Chloro-2,4-dinitrobenzene							
2-Mercaptobenzothizaole							
4-Phenylendiamine							
α-Hexyl-Cinnamic aldehyde							
Cinnamic alcohol							
Citral							
Cobalt chloride							
Ethylene glycol dimethacrylate							
Eugenol							
Imidazolidinyl urea							
Isoeugenol							
MCI/MI							
Methyl methacrylate							
Nickel chloride							
Phenyl benzoate							
DL-lactic acid							
Isopropanol							
Salicylic acid							
Sodium lauryl sulfate							
Xylene							

- DPRA和KeratinoSens能较好的互补
- DPRA的阴性检测率为86 %，KeratinoSens的阴性检测率为81 %，两者结合，阴性检测率为100 %

Bauch et al., Tox in Vitro 2012

●组合实验



须注意，如果蛋白反应和DC细胞激活，两者得到相反的结果，那么则需使用权重分析，从而做出判定。

●组合实验

与人体数据相比		灵敏度（%）	特异性（%）	准确性（%）
In vivo 标准	LLNA	96	81	90
单一实验	DPRA	89	82	86
	KeratinoSens	86	73	80
	h-CLAT	75	77	76
	U-Sens	75	100	86
组合实验（两个方法组合）	DPRA+KeratinoSens	100	64	84
	DPRA+U-Sens	96	82	90
预测模型	DPRA KeratinoSens U-Sens	93	95	94

通过表中将三种检测方式组合的预测模型结果，可发现，
其灵敏度为93％，特异性95％及准确性94％，相对单一实验都有提高。

●PPRA实验

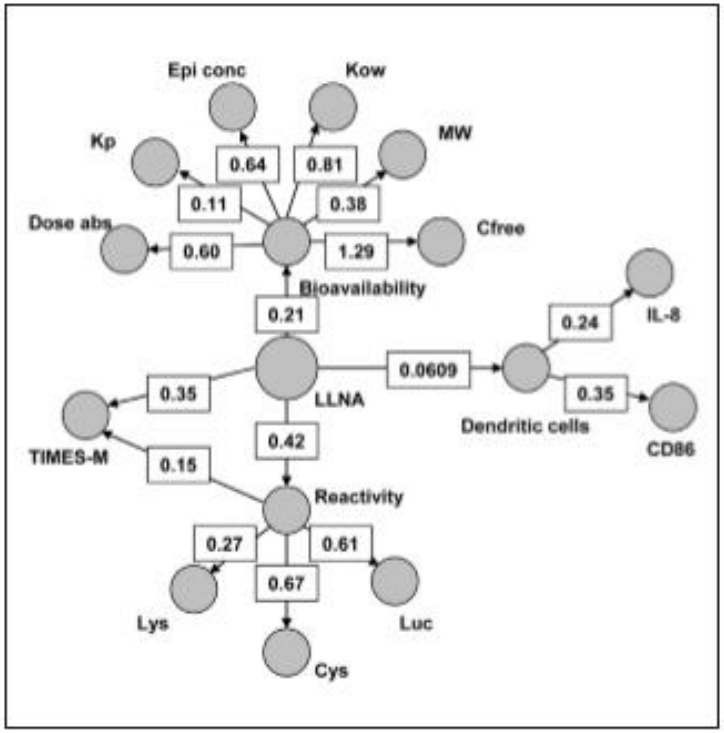
- Peroxidase Peptide Reactivity Assay (PPRA)
- 检测pre-hapten（空气氧化）和pro-hapten（酶氧化）
- 体系中加入horseradish peroxidase and hydrogen peroxide (HRP/P) 氧化体系
- 需用到HPLC/MS/MS

●整合策略—贝叶斯网络

Integrating Non-Animal Test Information into an Adaptive Testing Strategy – Skin Sensitization Proof of Concept Case

Joanna Jaworska[1], *Artsiom Harol*[1], *Petra S. Kern*[1], *and G. Frank Gerberick*[2]

[1]Procter & Gamble Eurocor, Strombeek-Bever, Belgium; [2]The Procter & Gamble Company, Miami Valley Innovation Center, Cincinnati, OH, USA



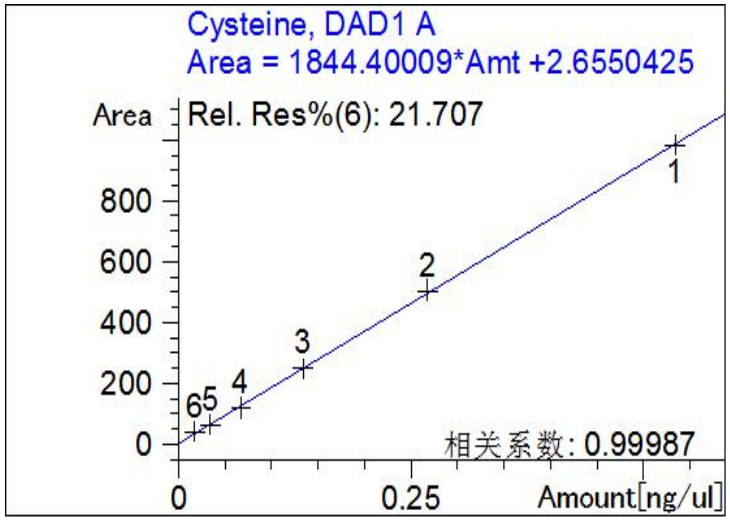
通过收集数据，包括物质表皮层生物利用度，DPRA试验结果，ARE试验，树突细胞激活等，利用Bayesian网络，将这些数据进行整合，从而得到该化合物在LLNA试验中的致敏潜力的可能概率值。

实验结果

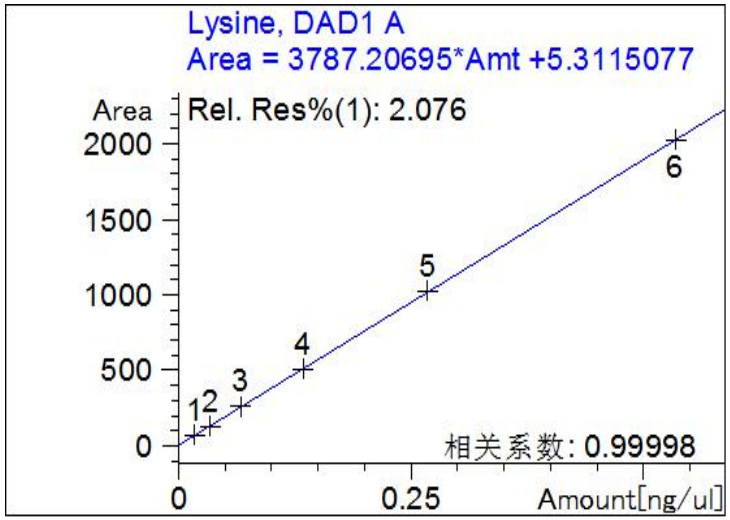
DPRA标准曲线

- 用0.534,0.267,0.1333,0.0667,0.0334,0.01667,0 mM绘制标准曲线
- 标准曲线r^2>0.990

Cysteine寡肽链

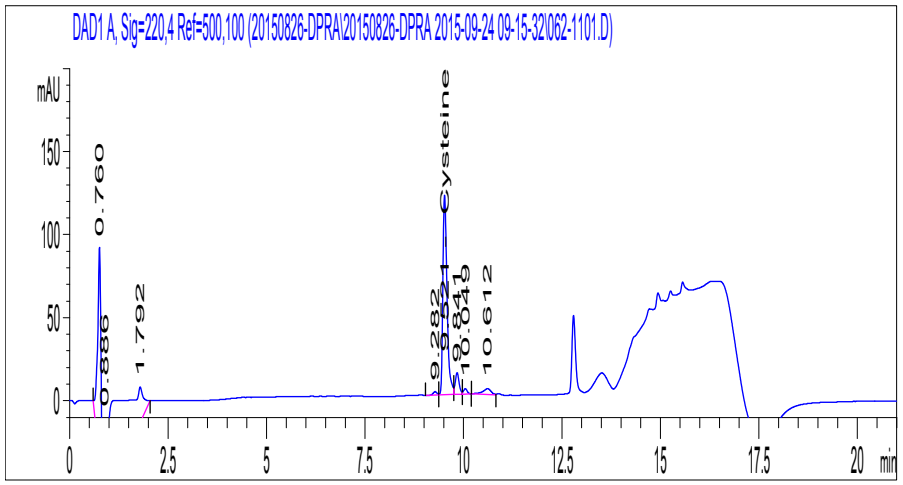


Lysine寡肽链



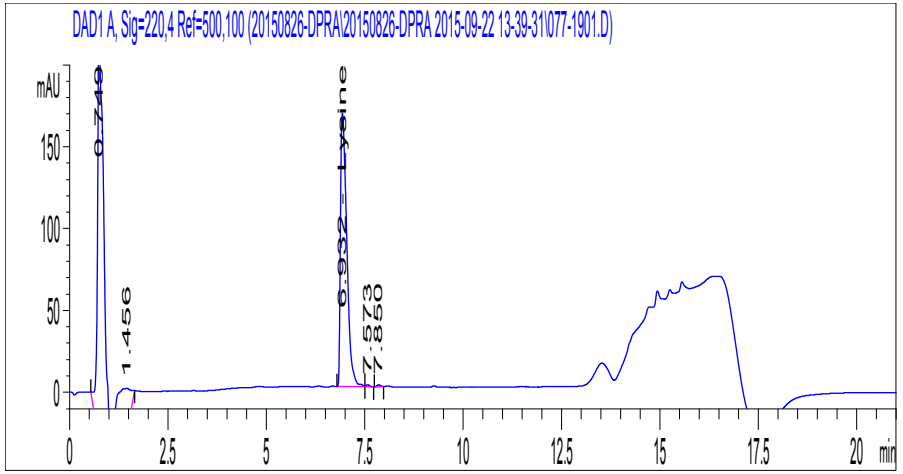
Cysteine

出峰位置：
9.521 min



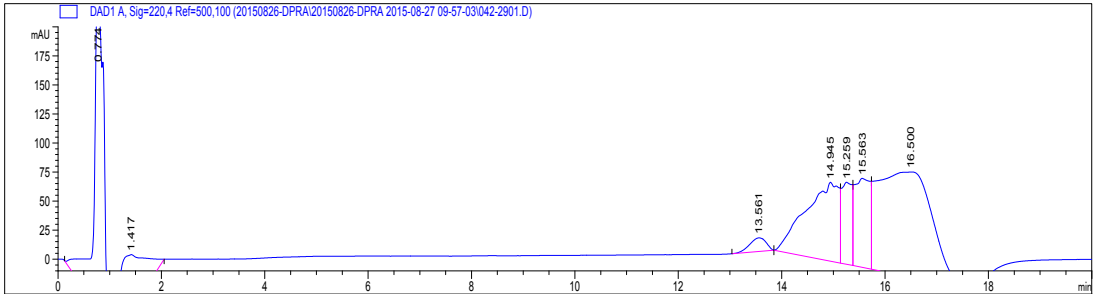
Lysine

出峰位置：
6.975 min



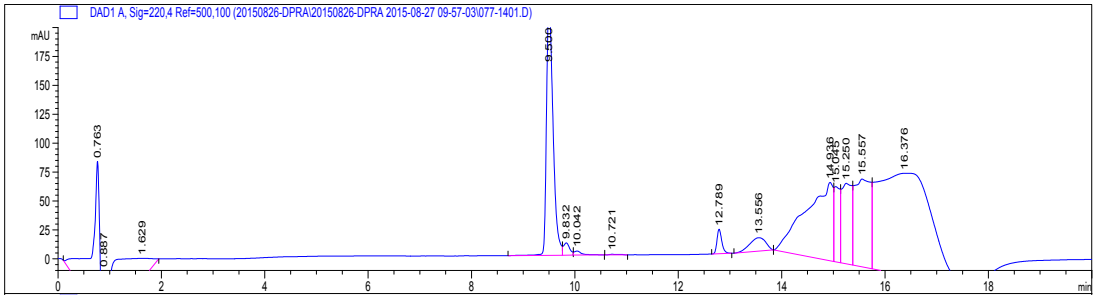
甘油

甘油

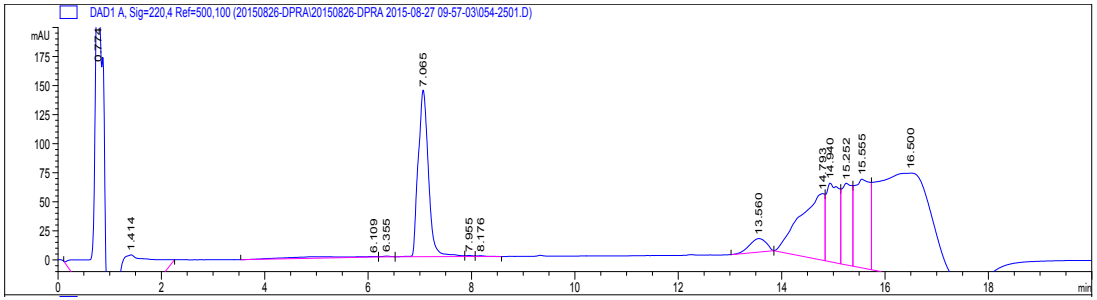


无Co-elution

甘油
+Cysteine



甘油+Lysine



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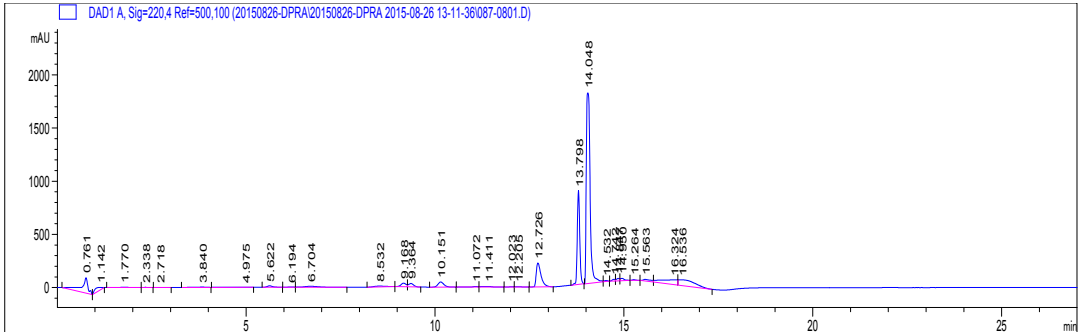
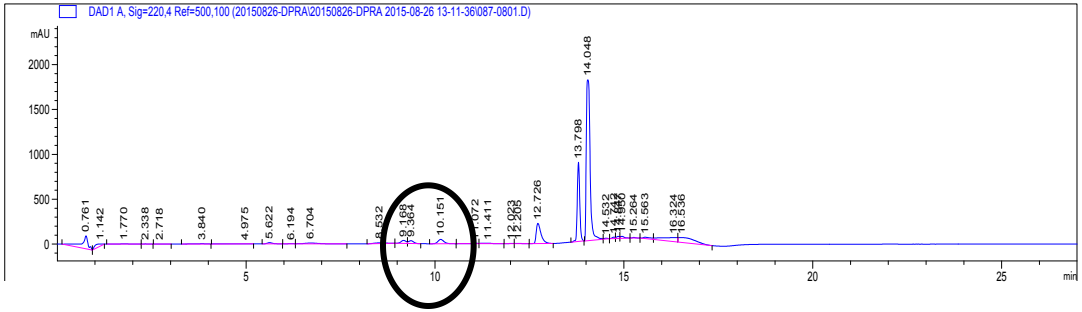


无Co-elution

肉桂醛
+Cysteine



肉桂醛
+Lysine