

# 化妆品安全与功效评价的整合策略

For a safer world

Integrated Test Strategy for  
Cosmetics Safety and  
Efficacy Assessment



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# 提纲 outline

## 一、整合测试策略概念和背景

Conception and background of Integrated Testing Strategy

## 二、安全评价的整合测试策略

ITS for safety

## 三、功效评价的整合测试策略

ITS for efficacy

## 四、应用前景展望

Prospect of application

## 一、整合测试策略的概念

ITS: 系列测试的组合，覆盖相关机制的步骤，并以合乎逻辑和假说驱动的决策程序组合，以有效利用产成的数据和获得用于全面危害或风险决策的信息。ITS can be described as combinations of test batteries covering relevant mechanistic steps and organized in a logical, hypothesis-driven decision scheme, which is required to make efficient use of generated data and to gain a comprehensive information basis for making decisions regarding hazard or risk.

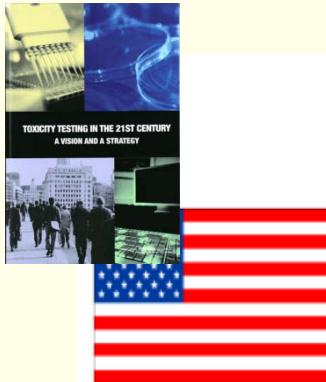
不同于分层方法tiered approach

- 决策是在最后一步，如皮肤刺激（OECD 2002）；
- 测试方法的排序是基于平均的生物这相关性；

# 大背景

科学驱动 science driven  
*Toxicity pathway—New strategies*

实用主义的替代法  
Pragmatism



## The Transatlantic Divide

Top-down development  
of new toxicological tools

Tox-21c



3Rs=Alternative

Bottom-up support to  
alternative methods and  
legislative pressure



政策/法规驱动 policy driven  
*Cosmetics/chemicals → EU/OECD individual methods*



# 3R替代与TT21C

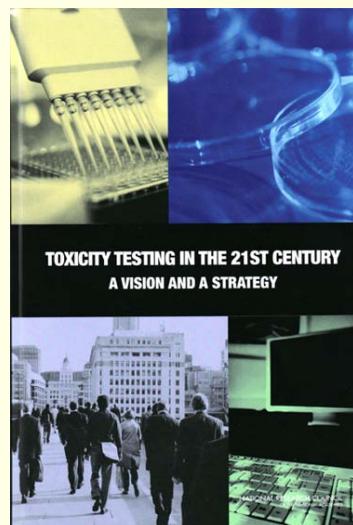
美国科学院报告（NAS Report）

--21世纪毒性试验：观念和策略

*Toxicity Testing in the 21st Century: A Vision and Strategy*

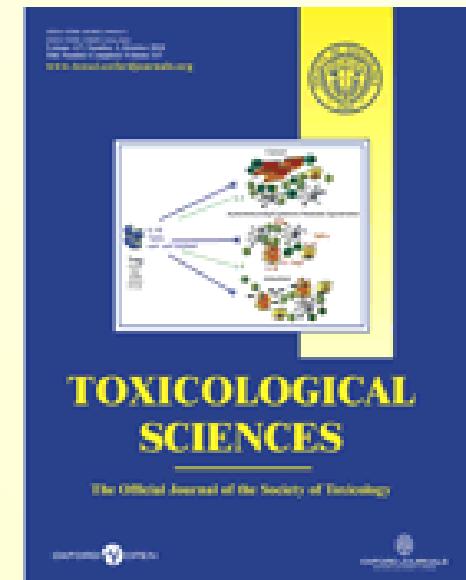
--科学与决策：推进风险评估

*Science and Decisions: Advancing Risk Assessment*

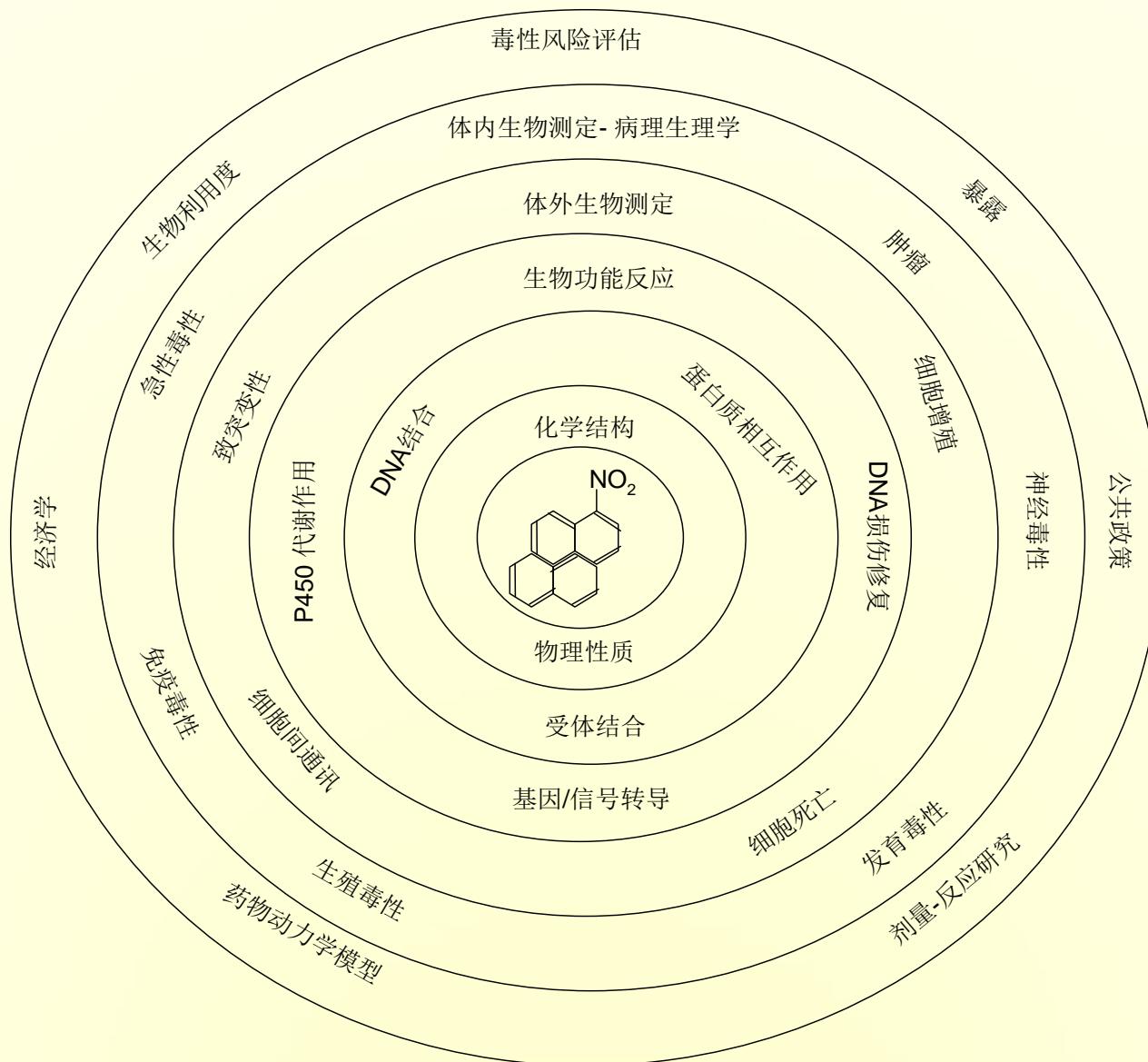


- 传统毒理学方法从1930建立
- 低通量，高成本
- 很少使用现代生物学技术
- 很少关注毒物作用模式
- 外推不确定性
- 与人群实际风险的相关性受质疑
- 大量使用动物

1. 使用人源细胞培养物  
系统生物学和通路的机制研究

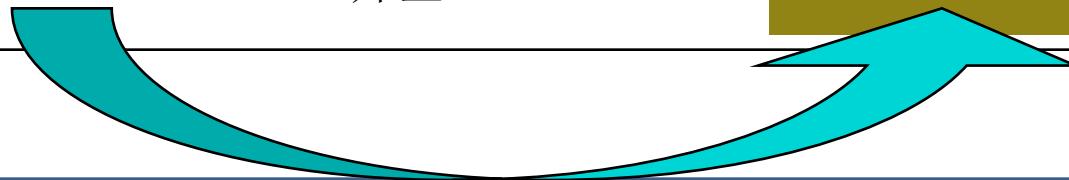


# 毒性预测的复杂性---单一 测试系统 局限性



# 化妆品整合测试策略的现状和未来

选择 I 体内实验	选择 II 分层体内实验	选择 III 体外/体内	选择 IV 体外
基于动物生物学	基于动物生物学	人体生物学为主	人体生物学为主
高剂量	高剂量	剂量范围广	剂量范围广
低通量	通量提高	高和中通量	高通量
高成本	低成本	低成本	低成本
耗时	少耗时	少耗时	少耗时
大量使用动物	较少动物	更少动物	几乎不用动物
单一终点	单一终点	毒性通路紊乱	毒性通路紊乱
	少量计算机或体外筛选	使用计算机筛查	计算机筛查



## 二、化妆品安全与功效的整合测试策略

Integrated testing strategy for cosmetics safety and efficacy

### 安全毒性实验

safety/toxicity test

**Eye irritation** 眼刺激: HET-CAM+BCOP+RBC+Epiocular  
**Skin irritation** 皮肤刺激: TER+  
皮肤模型  
**phototoxicity** 光毒性: 3T3 NRU  
**Skin Sensitization** 皮肤过敏:  
LLNA-BRDU  
**Percutaneous absorption** 皮肤吸收: *in vitro* 扩散池  
**Genotoxicity testing** 遗传试验:  
**Oral toxicity** 经口毒性:  
**cytotoxicity screen** 细胞筛选试验  
**embryotoxicity** 胚胎和发育毒性:  
**EST**  
**Endocrine disrupt** 内分泌干扰作用  
.....

### 功能功效实验

Efficacy test

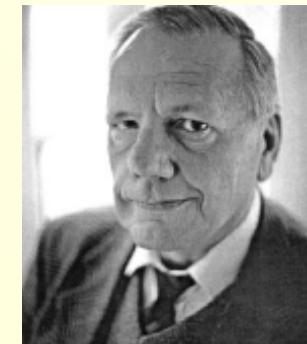
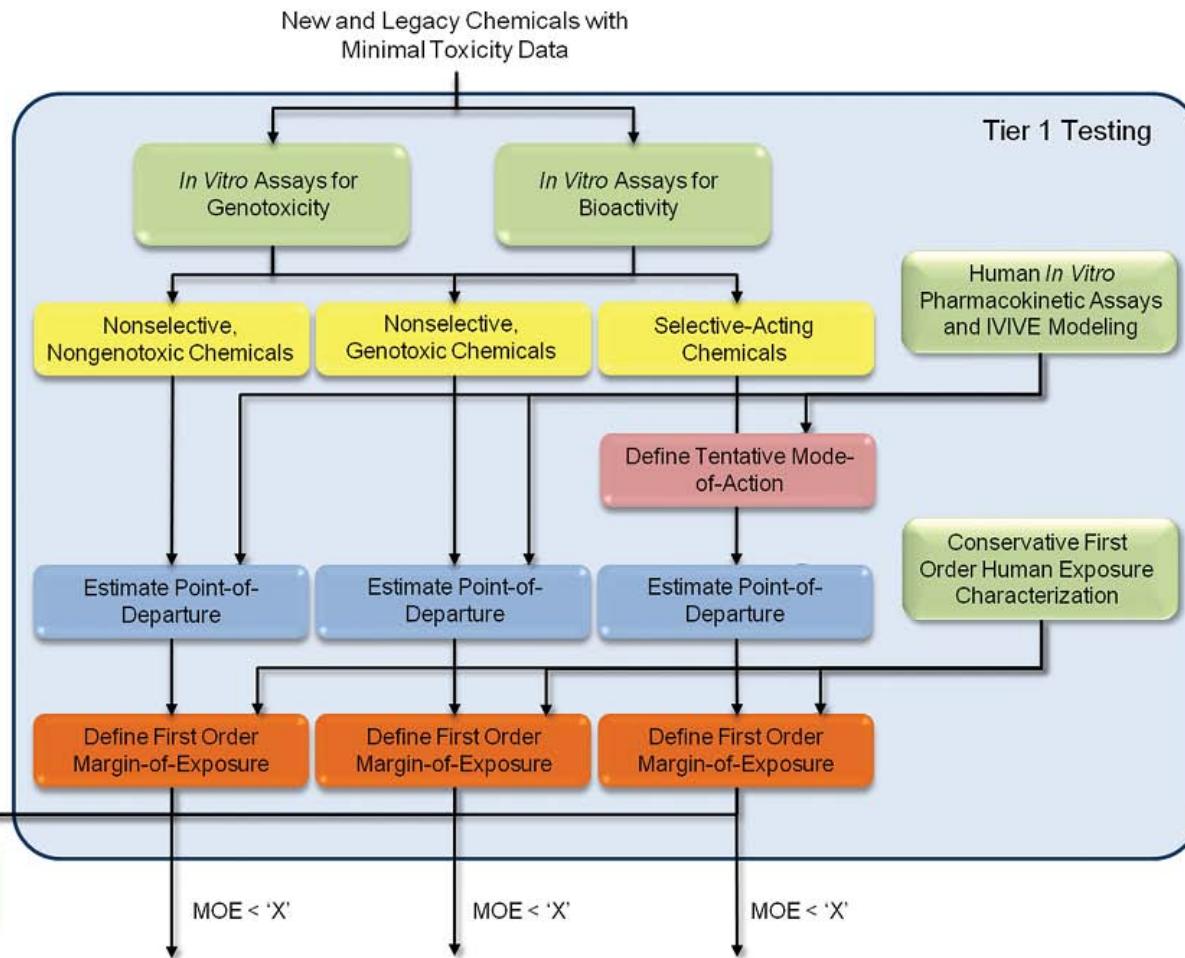
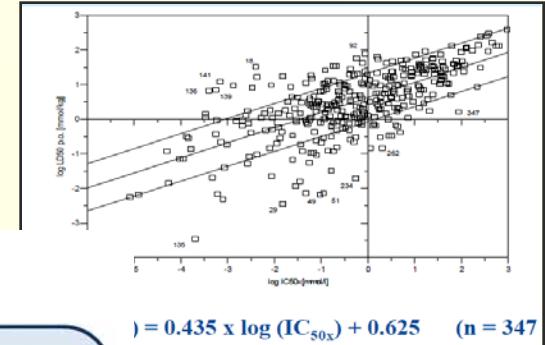
**Whitening** 美白作用  
**Nutrition** 营养细胞作用  
**Anti-oxidant** 抗氧化作用  
**Anti-ageing** 抗衰老作用  
**Anti-inflammation** 抗炎作用  
**angiogenesis** 促进血管生成  
**Hair growing** 生发育发作用  
**Anti adipose** 减肥作用  
**Basic cytomics** 基本细胞作用  
**Repair in vitro** 体外修复作用  
**Patch test** 斑贴试验  
**Photo patch test** 光斑贴测试  
**SPF test instrument assay and human test** 防晒指数测试  
(体外和人体测试)

适应性和功效测试

Adaptability and Efficacy test



# 细胞毒性及预测急性毒性



# 皮肤刺激的替代方法 Alternatives for skin irritation tests



**Transcutaneous Electrical Resistance  
Assay (TER) (OECD430)**

Positive threshold level ( $5\text{k}\Omega$ )



**Skin model testing 皮肤模型 OECD 431,439**

**EpiSkin, Epiderm**

# 眼刺激的整合测试策略

BCOP-437,ICE-438,FL-460

法规认可方法  
regulatory accept

有效未完成验证方法  
valid methods

IRE,RBC,CAMVA,HET-CAM,NRR

化学品chemicals  
日化产品household /  
化妆品cosmetics  
药品/农药  
medicine/pesticide

产品配方研发  
ingredients/formula  
终产品安全监控  
Products safety monitoring

科学scientific  
有效valid  
替代replace

投入成本  
COST

剂型form  
溶解性solubility  
颜色color  
固体/液体Solid/liquid

# 眼刺激的鸡胚方法

绒毛尿囊膜

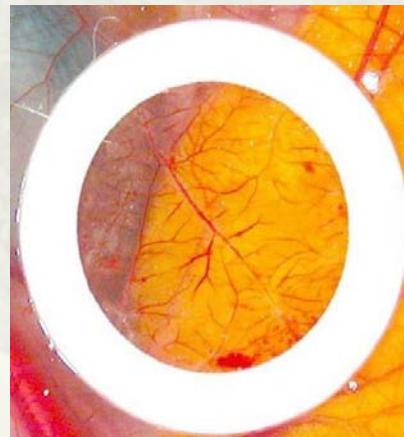
Chorio-allantois membrane (CAM)

**Hen's Egg Test Chorioallantoic  
Membrane (HET-CAM)**

鸡胚绒毛尿囊膜检测

**Chorioallantoic Membrane Vascular  
Assay (CAMVA)**

绒毛尿囊膜血管检测



应用举例

## 鸡胚绒毛尿囊膜血管试验（CAMVA）

**CAMVA法计算RC50值，判定区间如下：**

**RC50 > 3.0% = 无刺激性**

**RC50≥1.0%≤3.0% = 中度刺激性**

**RC50<1.0% = 轻刺激性**

如果试验物质明显含有酒精，刺激性/非刺激性的阈值最大可达30%-40%

**CAMVA方法的科学使用：**

- 用于轻度到中度眼刺激性的评估，
- 适用于原料和配方间的研究和比较，
- 产品眼刺激性预测，
- 损伤作用机制的研究。
- 可与其它替代方法组合提供产品全面信息。

**特别用于：配方及工艺优化，**

**原料和产品的质控，**

**原料筛查和安全浓度，**

# Bovine Cornea Opacity and Permeability

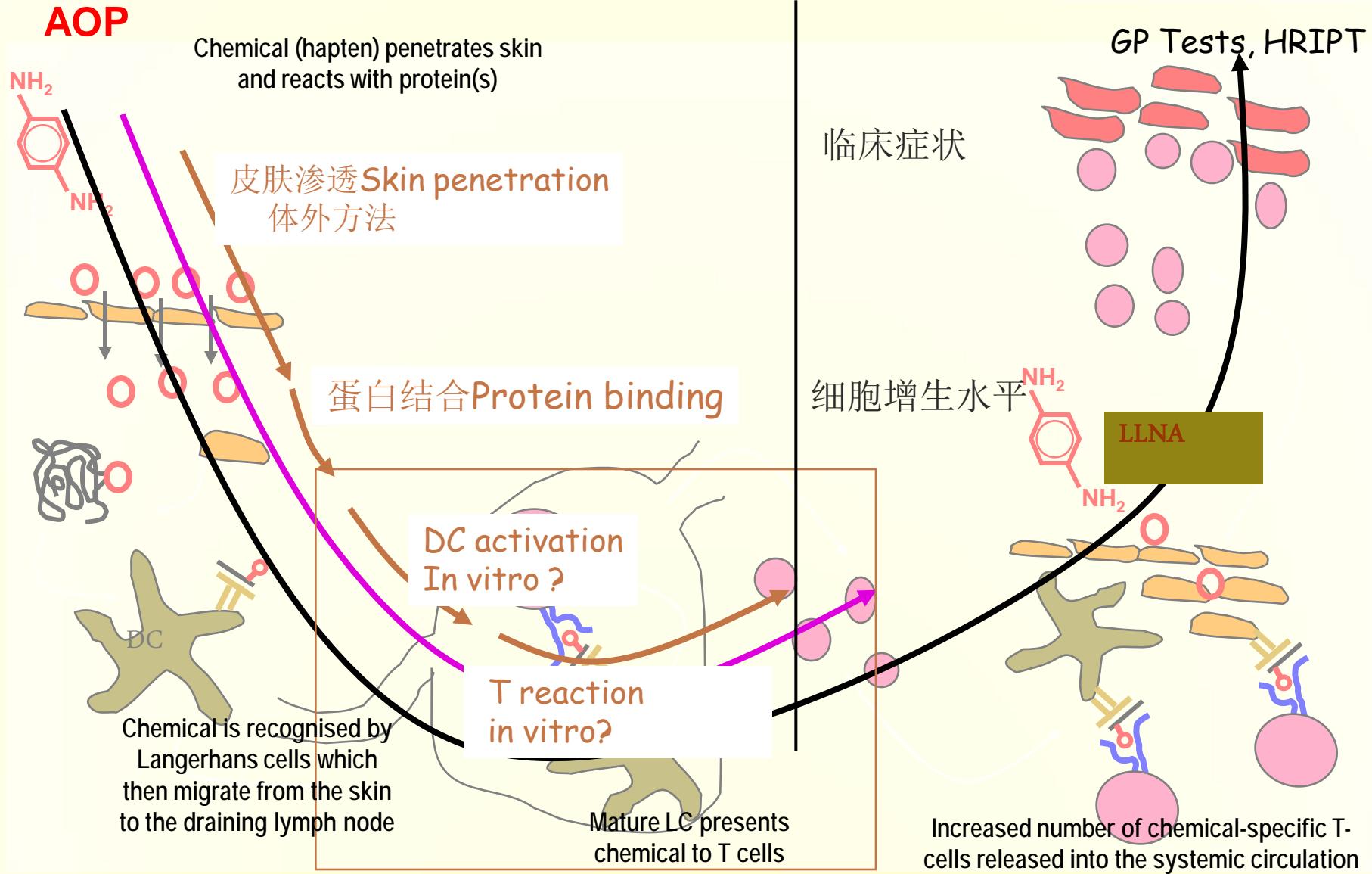
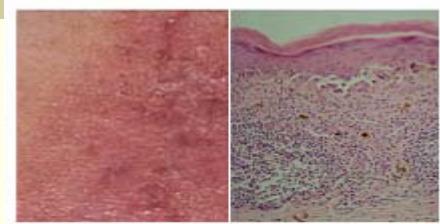
## 牛角膜混浊和渗透性试验 (BCOP)

### • 特殊试验规程 Specialized Protocols:

- 含表面活性剂：10%稀释，20或60分钟暴露，60分钟后暴露（重点关注渗透性分数）
- 多重暴露：原样，3和10分钟暴露，120分钟后暴露（适用于溶于有机溶剂的样品）
- 延长后暴露：10分钟暴露，4和20小时后暴露（适用于如  $H_2O_2$  的活性化学物）
- 组织学检查：可在任意试验后增加

### 应用举例

# 皮肤致敏实验替代方法 skin sensitization AAT methods



# 皮肤致敏的有害结局通路

## Adverse Outcome Pathway (AOP) for Skin Sensitization

分子特性  
Molecular properties

分子启动事件  
Molecular Initiating Event

细胞水平反应  
Cellular Response

组织水平反应  
Organ Response

机体反应  
Organism Response

Penetration  
into the viable  
epidermis

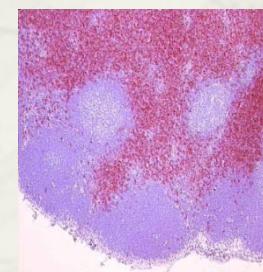
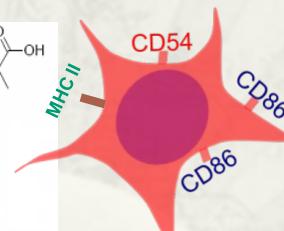
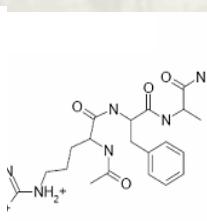
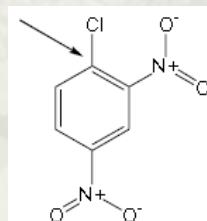
Electrophilic  
reactivity

Covalent  
interaction  
with proteins

Expression of  
cell surface  
markers and  
cytokines

Proliferation  
of T-cells in  
lymph nodes

Dermal  
inflammation  
(after  
challenge)



DPRA  
OECD 442C

+

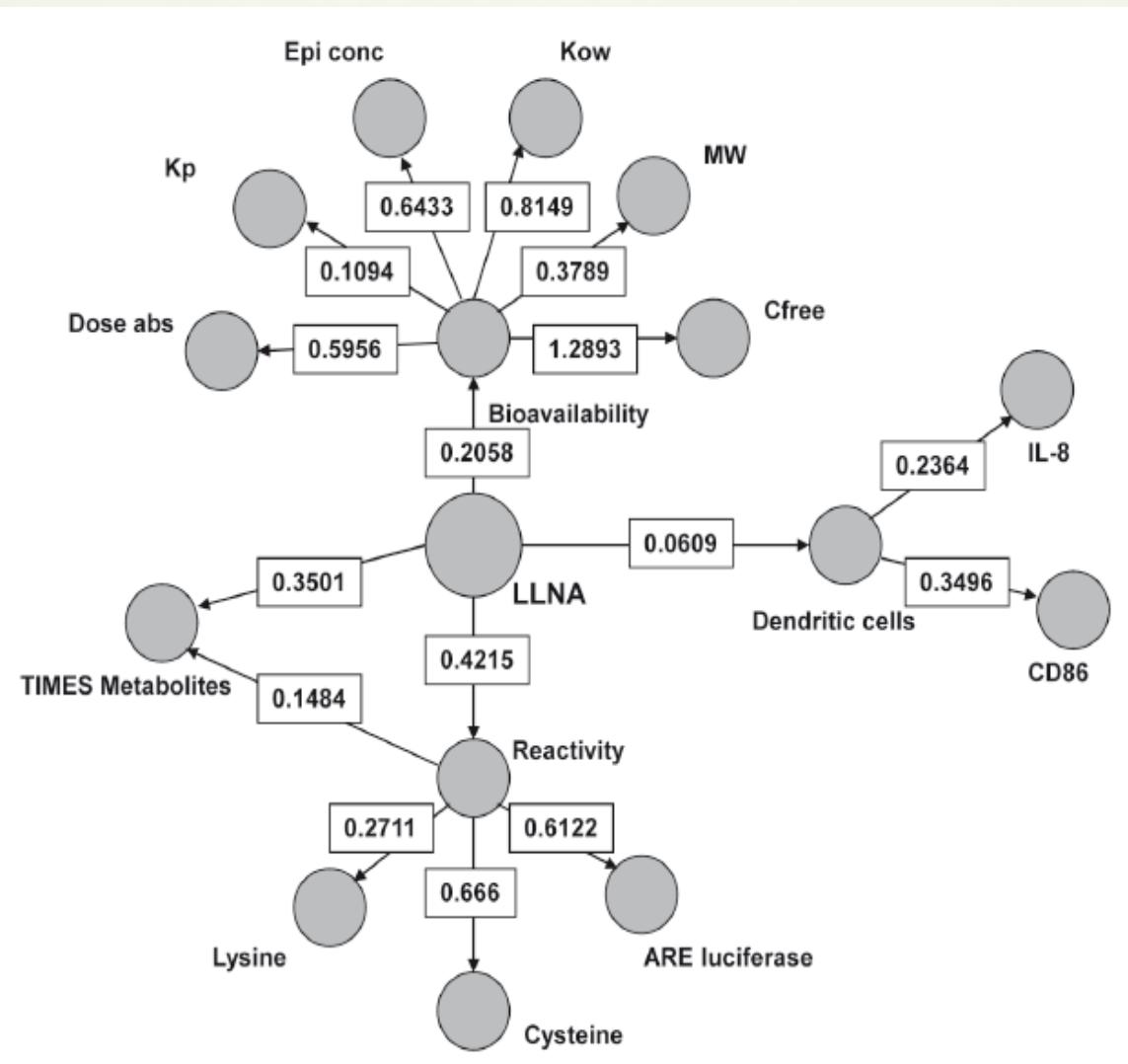
KeratinoSens  
OECD442D

h-CLAT

LLNA 442AB

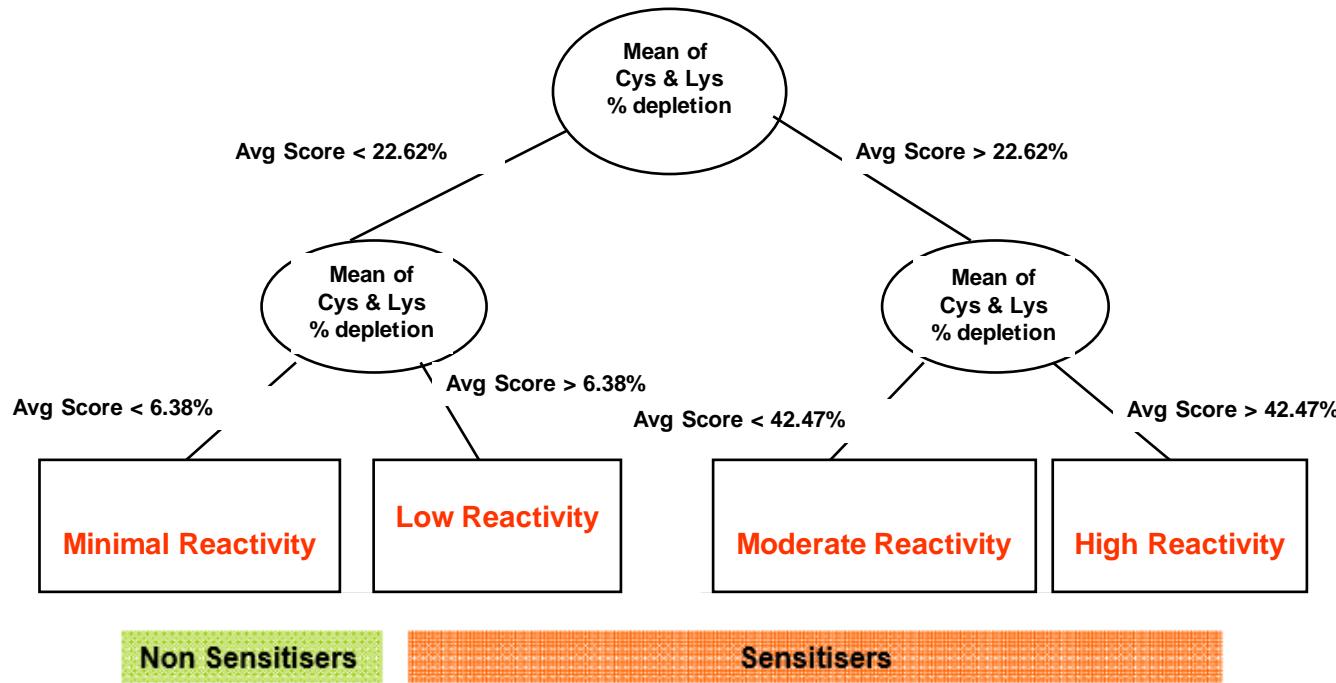
# In Vitro Test Strategy

- Direct Peptide Reactivity Assay (DPRA) OECD442C, 2015
- KeratinoSens, OECD442D, 2015
- 人细胞系激活试验 Human cell line activation test (h-CLAT)
- 骨髓U937细胞皮肤致敏试验 MUSST - Myeloid U937 skin sensitization test
- Development of AOP-based Integrated testing Strategy (ITS) for hazard identification
- OECD Test Guidelines on the 3 methods



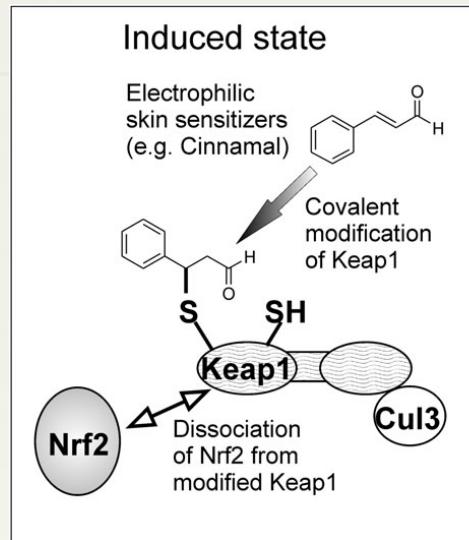
# 直接多肽结合反应DPRA: 预测模型

## Cysteine 1:10/Lysine 1:50 Prediction Model

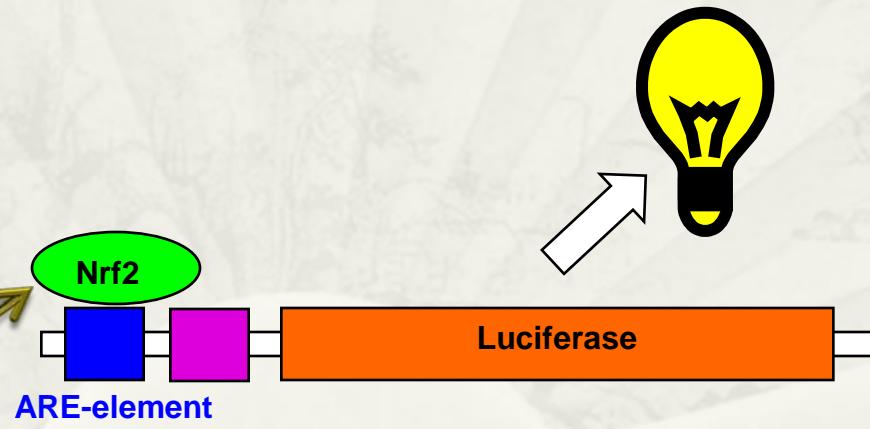
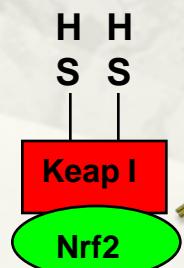
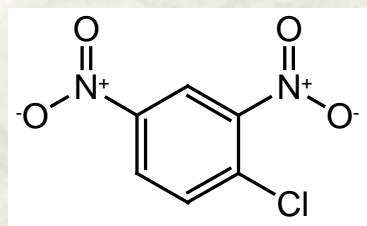
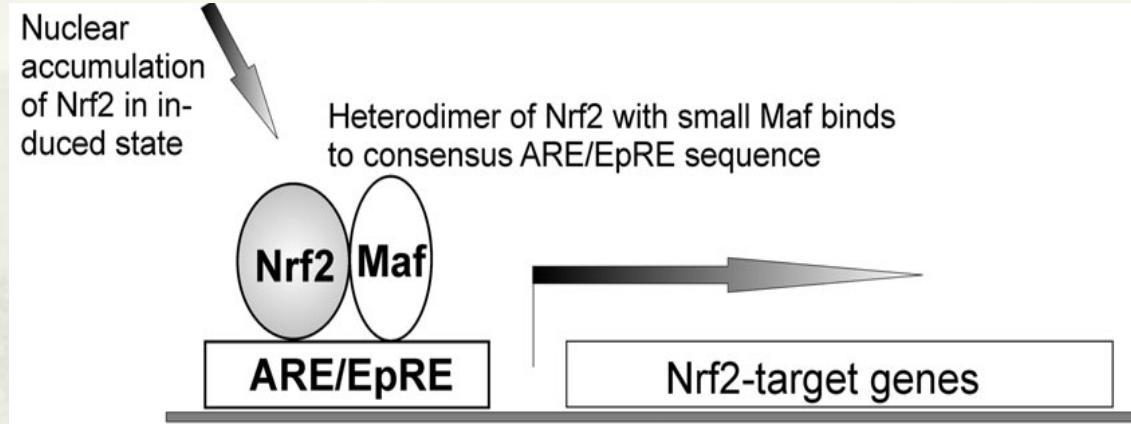


# 角质细胞致敏试验

## KeratinoSens Assay



- 原理: Nrf-2-亲电通路: Keap1蛋白+转录因子Nrf2+抗氧化反应元件(ARE)



- HaCaT 转染报告基因 (ARE-元件+荧光素酶基因)

# 人细胞系活化实验

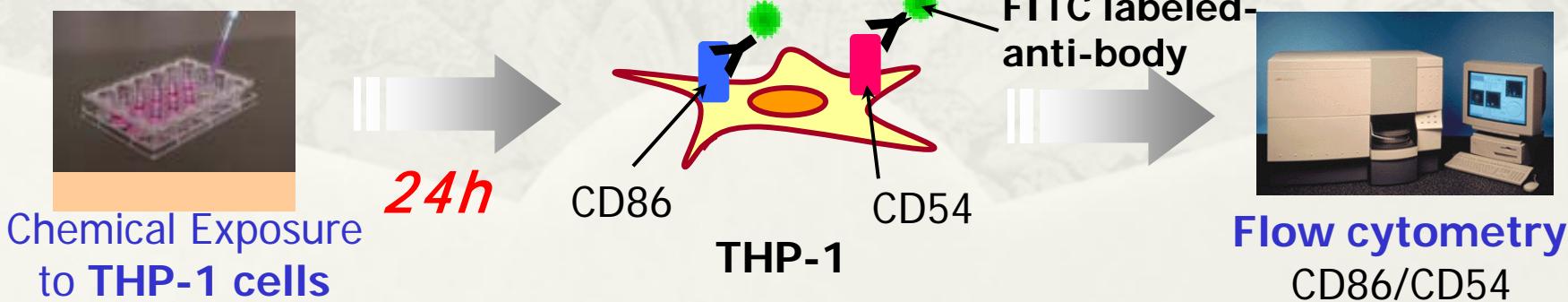
human Cell Line Activation Test (h-CLAT)

## ● Prediction Model

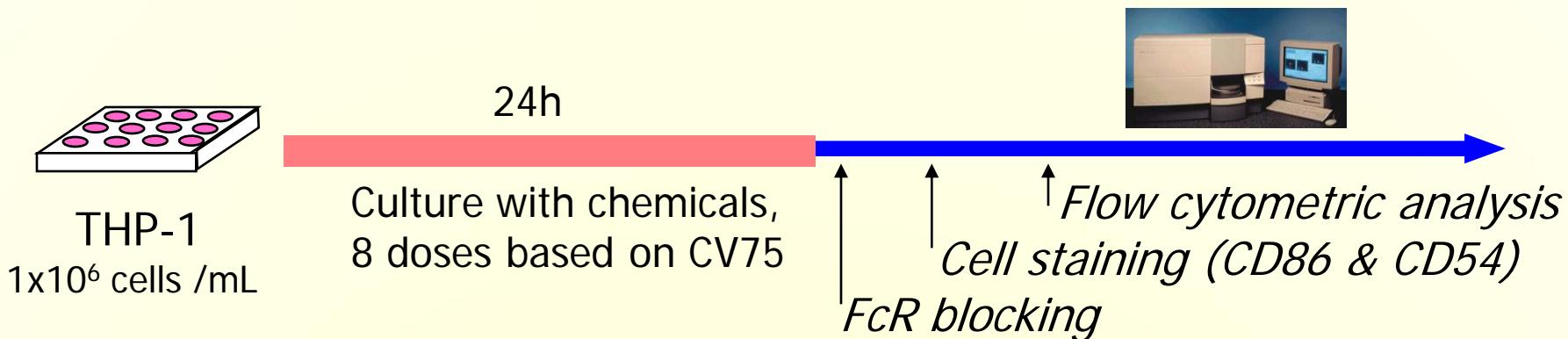
Viability  $\geq$  50% by Propidium Iodide (PI)

Positive criteria: CD86 RFI  $\geq$  150% and/or CD54 RFI  $\geq$  200%

Positive: 2 of 3 independent data at any dose should exceed the positive criteria



# Overview of h-CLAT



## ● Relative Fluorescence Intensity (RFI)

$$RFI = \frac{\text{MFI of chemical treated cells} - \text{MFI of chemical treated Isotype control cells}}{\text{MFI of vehicle control cells} - \text{MFI of vehicle Isotype control cells}} \times 100$$

\*MFI = geometric mean fluorescence intensity

## ● Prediction Model

Viability  $\geq$  50% by Propidium Iodide (PI)

Positive criteria: CD86 RFI  $\geq$  150% and/or CD54 RFI  $\geq$  200%

Positive: 2 of 3 independent data at any dose should exceed the positive criteria

\*: Ashikaga et al., 2006 Toxicol In Vitro 767-73., Sakaguchi et al., 2006 Toxicol In Vitro 774-84.

# 神经毒素体外替代方法

方法名称	试验系统	标准化	检测样品
促离子谷氨酸受体检测 神经毒性	大鼠和小鼠脑膜；转染细胞和 爪蟾卵；原代神经元培养	检测软骨藻素的方法已标 准化	所有化学物、神经毒性、海 洋生物样品
GABA <sub>A</sub> 受体检测神经毒性	大鼠和小鼠脑组织和神经原代 培养，表达GABA <sub>A</sub> 受体的工程 细胞系和爪蟾卵	优化中，积累数据以应用 于组合筛查	有机氯杀虫剂、惊厥药、驱 虫药阿维菌素、毒蝇鹅膏菌 毒等。苯环类、巴比妥类药 物和麻醉剂等
电压敏感钠离子通道检 测神经毒性	脑组织；原代培养大鼠或小鼠 神经和肌管；人或啮齿类神经 细胞系；表达神经钠离子通道 亚单位的爪蟾卵	正进行室间转移，美国FDA 已批准用于确证雪卡毒素， 正在等待AOAC认可为替代 小鼠生物试验的方法	化学物、农药、神经毒素、 贝类毒素、蝎毒素，杀虫剂
神经细胞突起生长形态 学测定和生化试验检测 轴突病变神经毒性	外植体培养、源于成神经细胞 瘤和神经胶质瘤的细胞系，神 经元	开发和优化中，需要积累 基础数据	有机磷酸酯类化合物、工业 化学物、有机磷酸酯类药物、 抗肿瘤药物
神经酯酶试验体外筛查 有机磷化合物神经毒性	大脑细胞重聚团；大脑匀浆； 人、大鼠和小鼠神经细胞系	已在多个实验室使用，实 验室间一致性较好。	有机磷类和氨基甲酸酯类杀 虫剂、农药和工业化学品、 有机磷类药物制剂
神经细胞乳酸脱氢酶 (LDH) 释放试验	原代培养细胞（神经元和星形 胶质细胞）	常规试验方法	工业化学物，神经毒素
神经毒性试验的脑细胞 聚集培养高内涵方法	大鼠脑细胞聚集培养	已开发和用于研究实验室 /ACuteTox项目开发	急性神经毒性评价，

# 神经毒性的体外方法

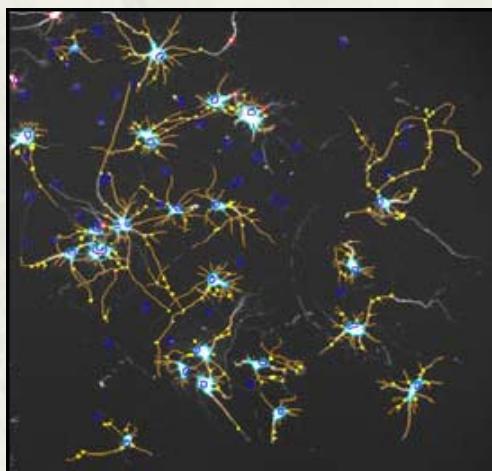
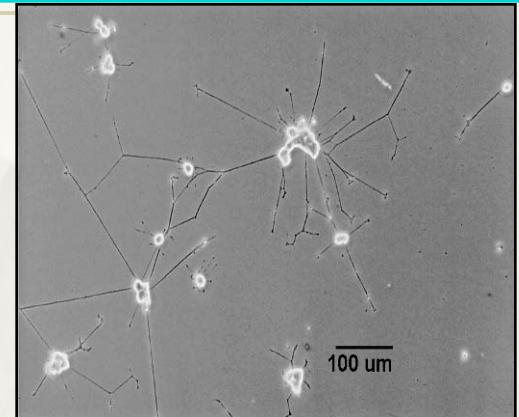
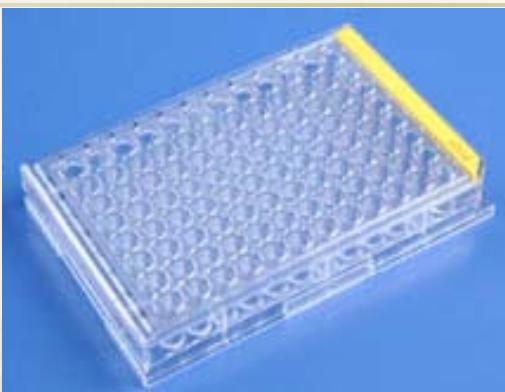
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- \* 体外方法依据脑发育过程中的关键事件
  - \* 增殖、分化、生长、突触形成、髓鞘形成、凋亡
- \* 检测终点可以应用高通量/高内涵技术
  - \* 细胞水平的检测点，生物标志物，分子信号
- \* 便于从已知的神经毒物建立训练集，提高检测方法预测未知毒性的能力
  - 为进入下一步测试的先后顺序提供数据

# 基于高内涵的神经毒性检测技术 High Throughput Cell-Based Assays

## High Content Analysis

- 高通量成像
- 细胞水平数据
- 非常适合神经毒性的研究和检测

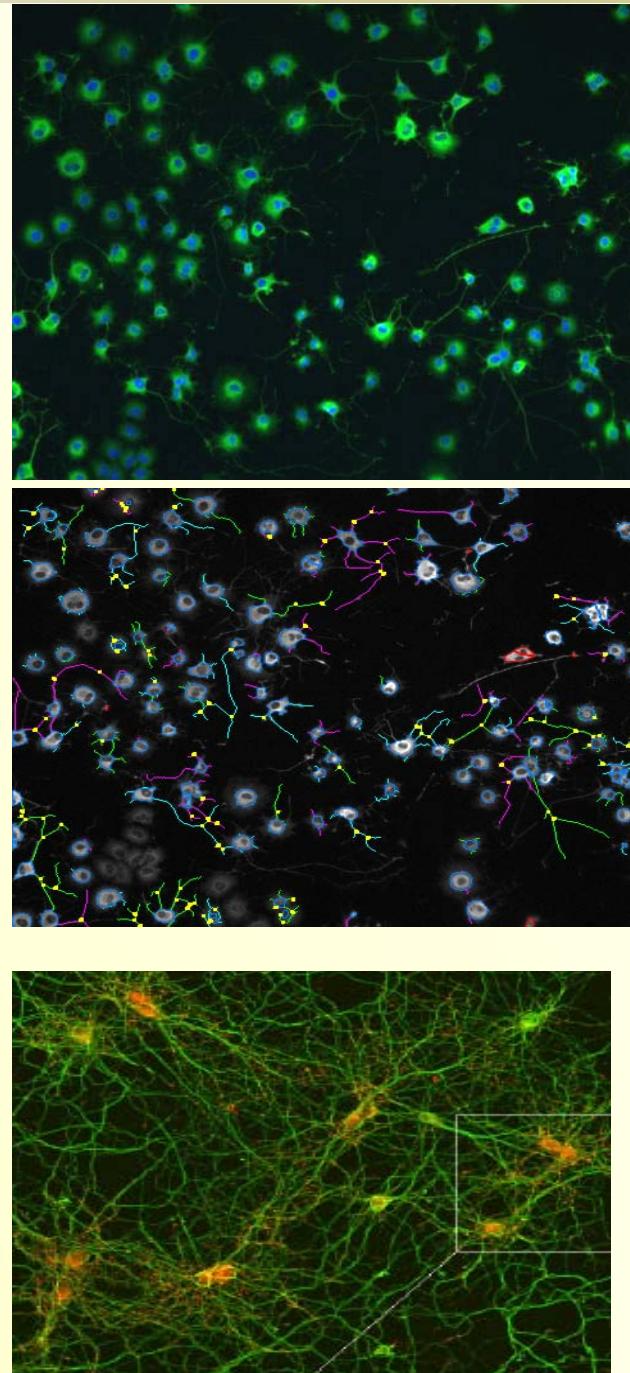
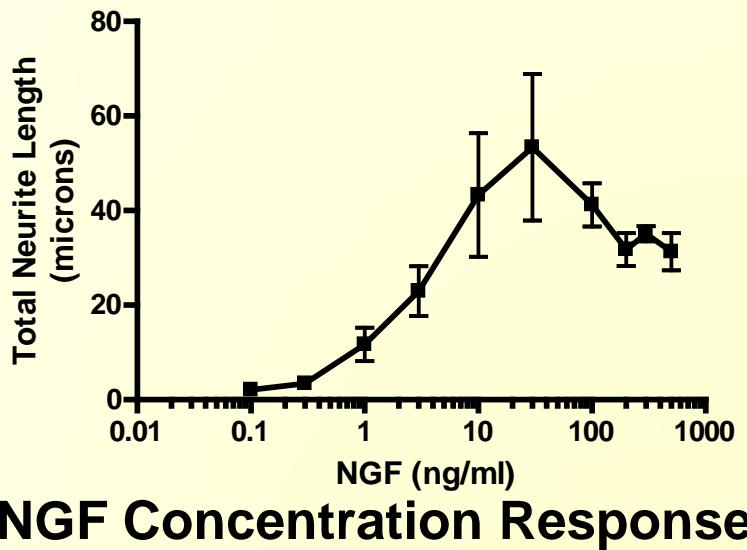


输出: 自动分析96孔板, 耗时20-30 min  
(200 细胞 x 96 孔 x 10 终点/细胞 = 192,000 数据)

# 原代神经细胞或细胞系的检测：轴突生长

## NS-1 Cells (Clonal PC12 cells)

- 检测化合物刺激/抑制轴突生长作用（如NGF）
- 4天后固定和染色
- 30 min自动检测
- 多种靶标的荧光标记：CY3突触素，FITC $\beta$ -微管蛋白，DAPI



# 神经毒性检测方法的验证

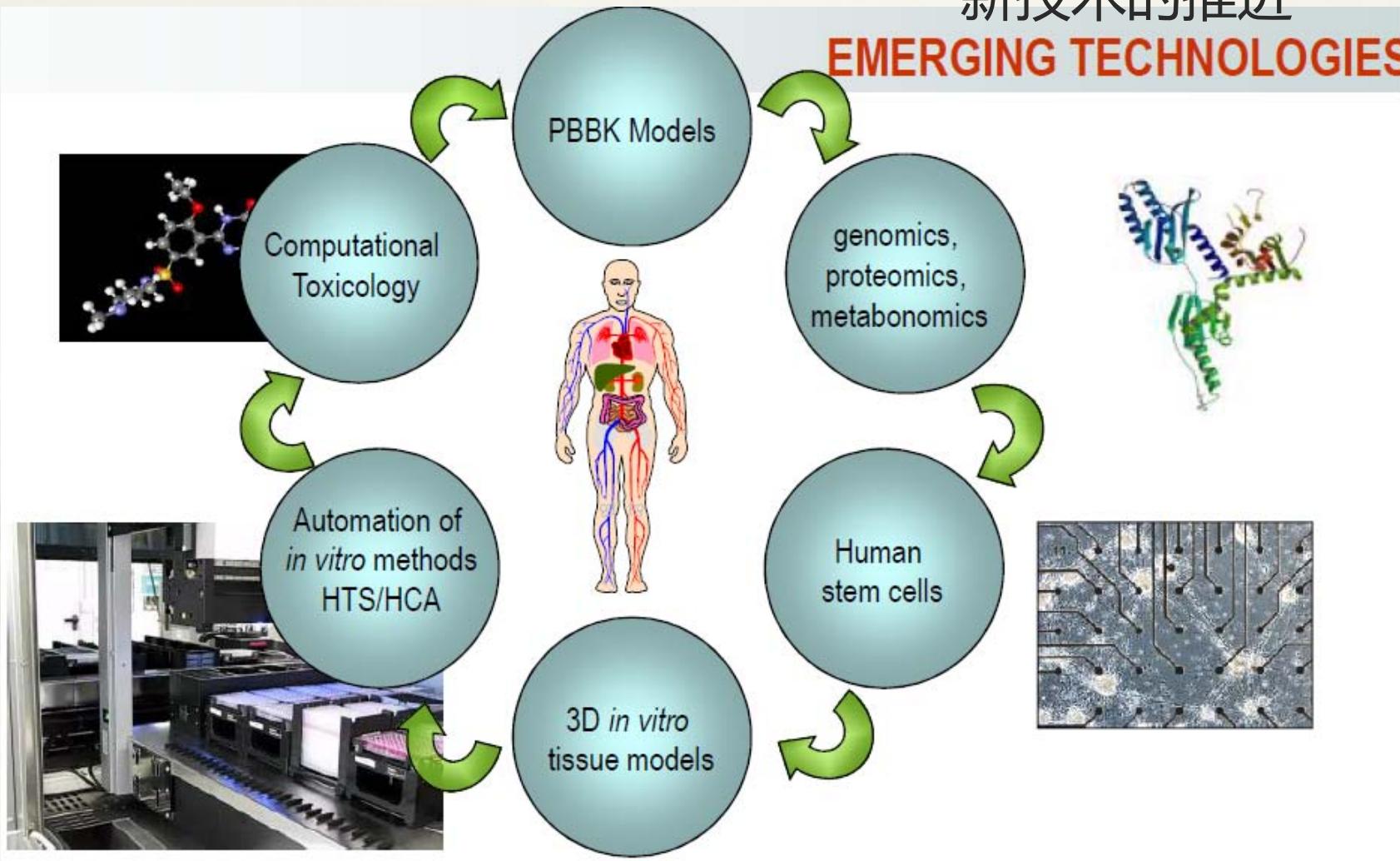
- \* 建立神经毒性参考物质库神经毒性?  
发育神经毒性?  
相同机制?  
复合机制?  
种属差异?
- \* 验证的模式  
传统验证?  
模块验证  
证据权重?  
组合策略?

Human and Animal Data	Only Animal Data
amphetamine chlordiazepoxide cocaine diphenylhydantion hexachlorophene ethanol lead methylmercury PCBs terbutaline thalidomide trans-retinoic acid	acrylamide benzene cadmium chlorpyrifos (oxon) dieldrin ketamine methadone methamidazole methanol methylazoxymethanol propylthiouracil trichloroethylene triethyltin trimethyltin valproic acid

# 技术创新无止境

新技术的推进

**EMERGING TECHNOLOGIES**



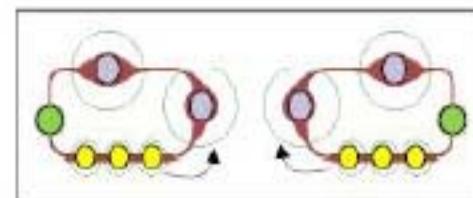
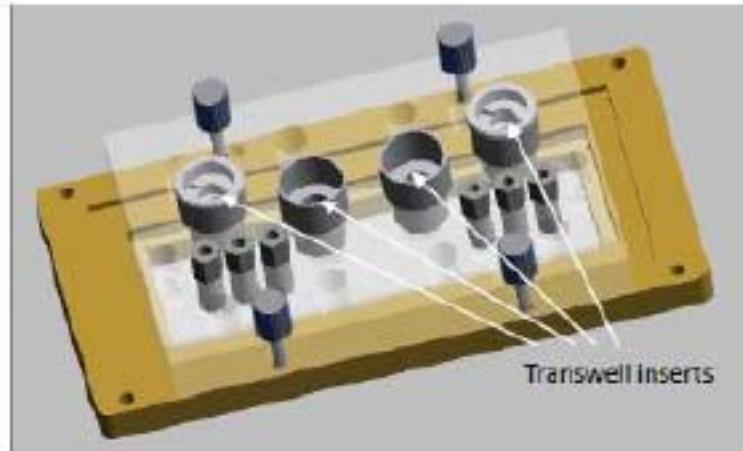
# Pathway Testing – toxicogenomics, etc.

## The Multi-Organ-Chip (MOC) technology



Laboratory bioreactor

- two peristaltic micro-pumps per chip
- two media perfusion circuits per chip
- adopted to use of Transwell-inserts
- two MOC's (4 circuits) per bioreactor



MOC's of microscopic slide-format

- - peristaltic micro-pump
- - Transwell inserts
- - injection port

# Innovation alternatives methods

## 2015-2016

1. Integrated testing strategy for skin and ocular irritation
  2. AAT for children skin care products
    - more rigorous evaluation and classification standard
    - more specific testing endpoint and time  
IL-1ra/IL-1a, TRPV1 (VR-1) +Ca 2+
  3. AAT anti eczema of health care products
    - CD4+ T Th-2
    - sensory neuron Ca2+ mobilization and CGRP release
  4. Oral Irritation and Anti-inflammation predictive
    - related to BCOP model
    - K3/K67,P63 biomarker
    - imagine fluorescence
- .....

## **Standardization**

- OECD TG  
431,432,437,439,442A,442B,442C,  
460
- ISO 10993.5-10,
- SN/T 2285, 2328, 2329, 2330,  
3898, 3899
- GB/T 21769, 21757, 21804, 21826,
- ECVAM DB-ALM: INVITTOX n  
3,47,54,78,96,100,109,108,113,127  
,139,...
- ICCVAM/ NIH: No 02-4502, 04-  
4510, 07-4618

+

## **Customization**

- Ocular: CAMVA,  
CAMVA+BCOP
  - Skin Irritation: Monolayer cell  
+3D Skin Model
  - oral mucous membrane  
irritation
  - Establishing an in vitro  
screening test for developmental  
neurotoxicity
  - Personal care products in vitro  
test based
  - TRP channels family at infant  
skin development and  
mechanism of mediated skin  
sensitization
  - Anti-irritation study by 3D skin  
model

# In Vitro Skin irritation/anti-irritation

In Vitro Skin Corrosion-MTT (Episkin-C)

In Vitro Skin Irritation-MTT (Episkin-M)

In Vitro Skin Irritation -MTT+ILa (Episkin-I)

Keratinocyte testing for skin irritation for ingredients – cytokines

In Vitro Skin Irritation Inhibition Assay by skin model-MTT+ILa (SDS)

In Vitro Skin Irritation Inhibition Assay by skin model-MTT+ IL-8+PGE2 (PMA)

Keratinocyte anti chemoline/ cytokine assay(IL-8\CXCL-1\PGE2)

Antimicrobial peptide assay  $\beta$ -defensin release

# In Vitro Efficacy Testing

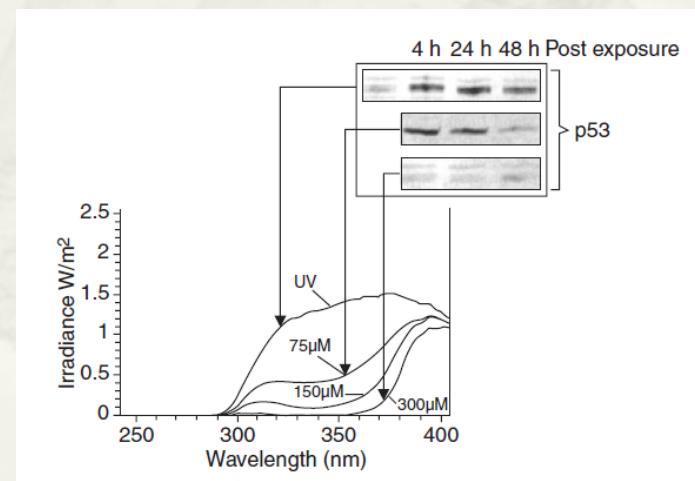
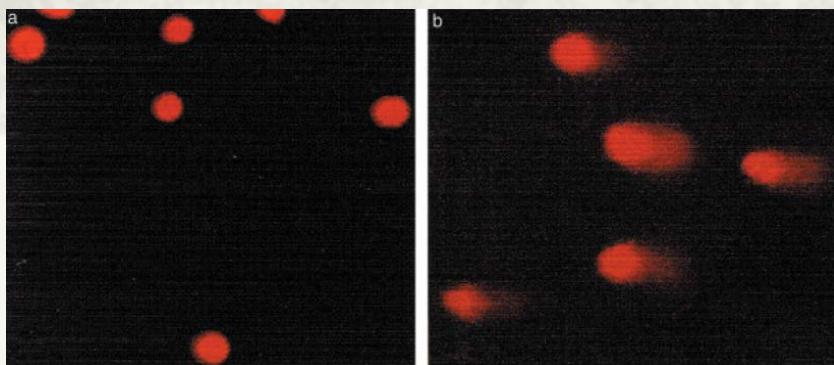
## Epidermal Protection Against UV and free radical

NHK anti-oxidant assay induced by UVB--MTT, lipid peroxide (C11-Fluor probe)

NHK anti-oxidant assay induced by UVB or chemical ---JNK1/2 phosphorylation

Skin model(Episkin) sunburn cell assay-histology

3D Human skin model anti-oxidant assay induced by UVB or H<sub>2</sub>O<sub>2</sub> -- MTT,MDA



# In Vitro Efficacy Testing

## Keratinocytes, structure and function of epidermal barrier

NHK migration or proliferation -imaging and /or marker expression

Epidermis to dermis migration-imaging

NHK differentiation-- marker expression and ECIS

Skin model(Episkin) differentiation--marker expression

NHK or Skin model/ Episkin Hyaluronic acid synthesis or hyaluronidase inhibition

Proteoglycan of ECM by NHK or Skin model/ Episkin by fluorescence

Cell adhesion and cell junction by NHK or Skin model/Episkin--fluorescence

## Anti-aging assay

Aged fibroblast prevention by inhibition  $\beta$ -galactosidase

Aged fibroblast tropoelastin synthesis

Aged fibroblast ECM assay or procollagen synthesis by staining

MMPS release assay by chemicals stimulation

# 紫外线光损伤的生物学

## 美白剂筛查的整合测试



阻止紫外线

1

Chamomilla ET,  
氨甲环酸

阻止炎症因子产生  
抑制黑色素细胞活化

黄芩根



Inhibition of ET-1 production

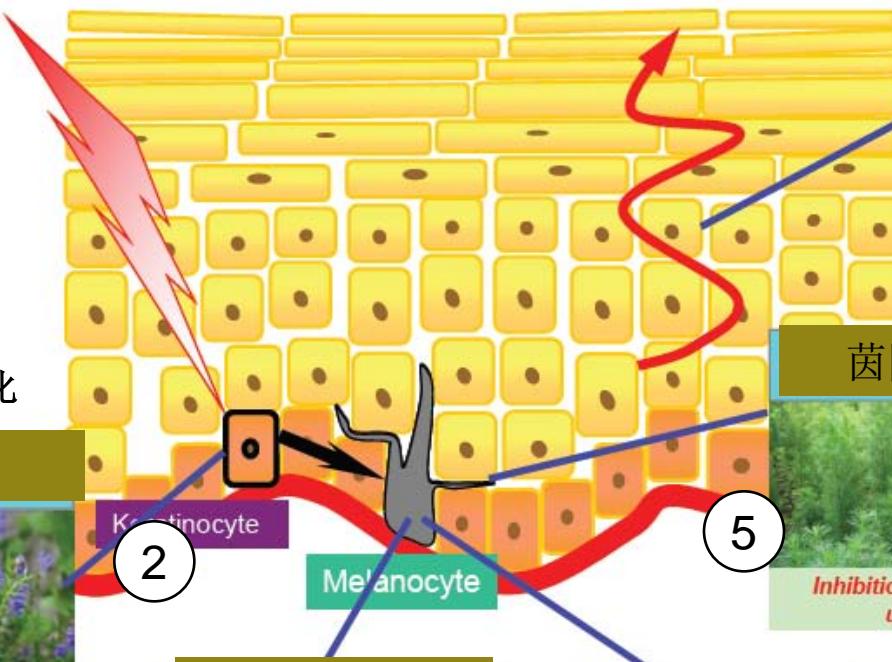
CHANEL PRECISION  
Blanc Essential series



抑制黑色素合成

抑制Tyrosinase,TyRP1,促进分解

熊果苷、曲酸、鞣花酸、亚油酸, Magnolignan, 甲氧基水杨酸钾



促进角质剥离  
表皮周转/黑色素排出

枣果



Promotion of skin turnover



茵陈蒿花

Inhibition of melanin uptake

抑制黑色素小体  
抑制黑色素小体成熟-转  
运-传递

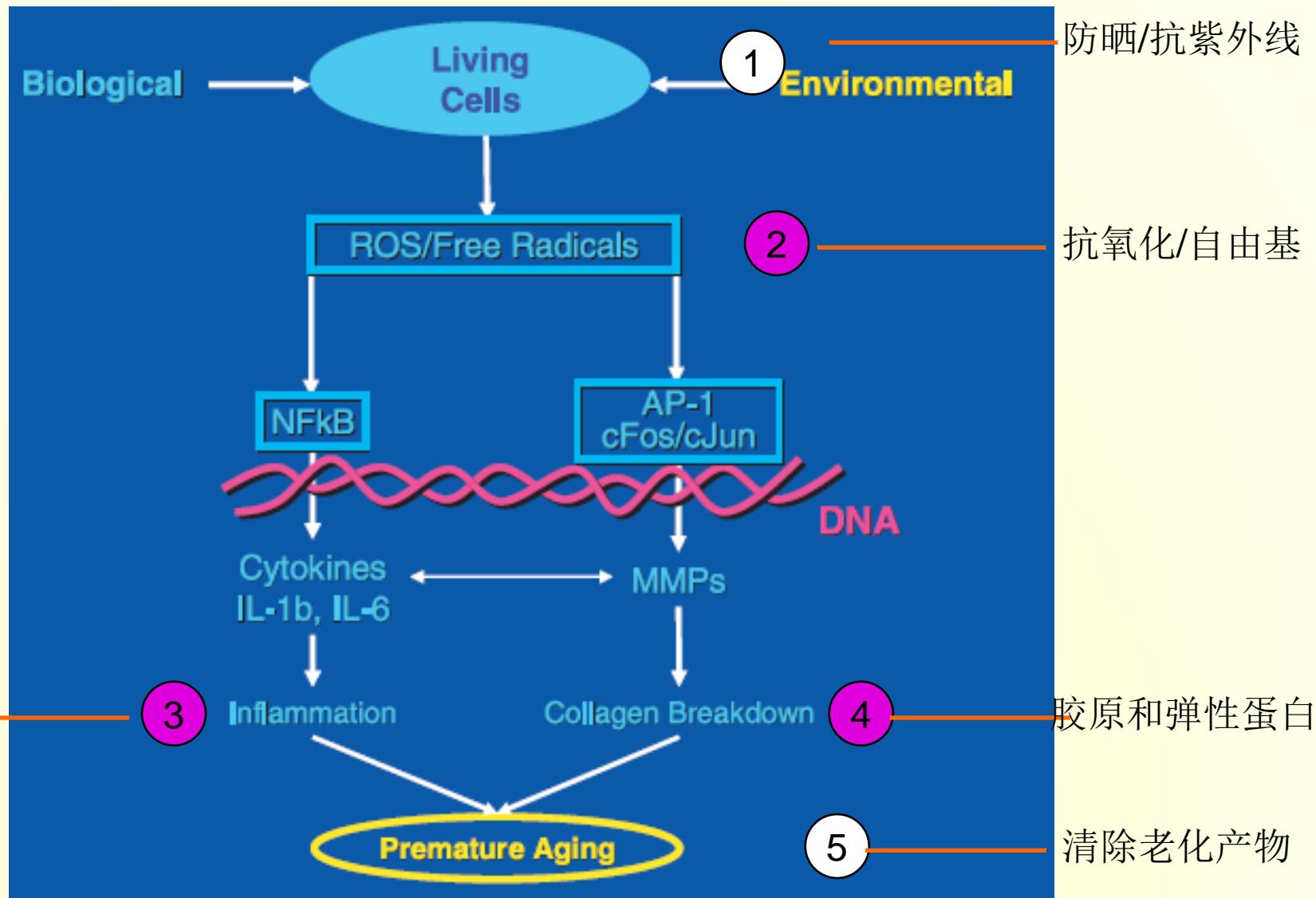
4 白皮



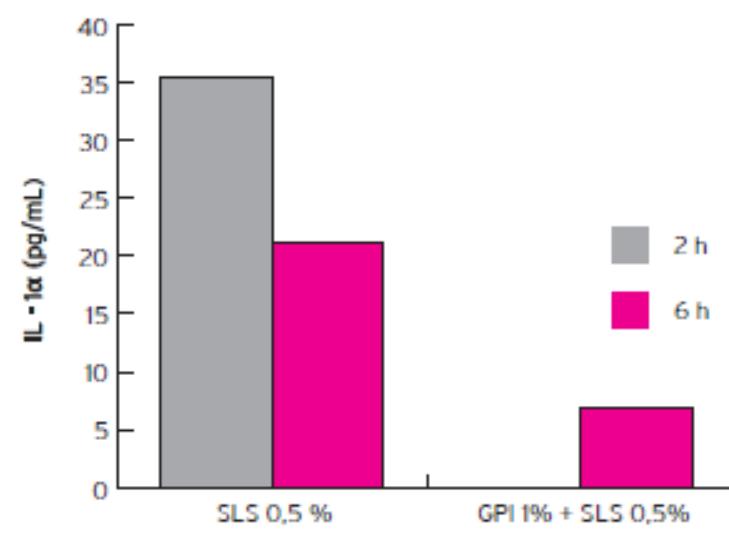
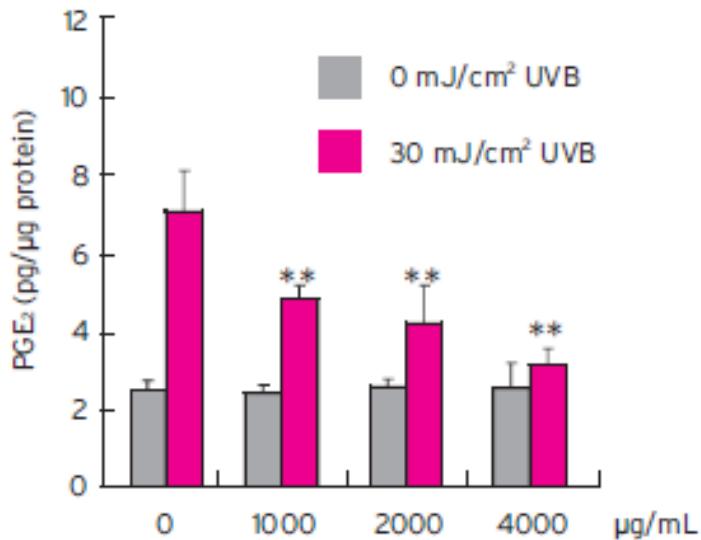
Inhibition of melanin production

OLAY (P&G)  
Natural White Healthy Fairness

# 皮肤细胞老化过程及整合测试



# 抗炎症试验 *Anti inflammation assay*



Keratinocytes were pretreated with different concentrations of test substance for 24h, then exposed to UVB, PGE<sub>2</sub> secreted were quantified by ELISA.

Test substance with or without SLS is applied for 24h on Episkin. After exposure, collected medium and assay the IL-1 $\alpha$  by ELISA.

# 要创新不要炒作

上海某高校2009年硕士论文：“化妆品安全性评价中皮肤毒性若干替代实验方法的研究”

----通过构建重组人**EpiDerm**皮肤模型，建立检测化妆品的皮肤腐蚀性/刺激性的替代实验方法

河北某高校2011年硕士论文“利用体外构建的组织工程皮肤模型进行皮肤刺激性检测的相关研究”

----根据欧洲替代实验确认中心20种物质的检测结果显示两种模型都适用于进行皮肤刺激性检测，而**利用永生化细胞构建的皮肤模型**的敏感性和特异性均高于利用原代培养细胞构建的皮肤模型。

2013年河北某高校硕士论文“皮肤模型的构建及在检测药物化妆品中的应用”

----本研究获得主要结果包括构建了皮肤表皮模型；**模型验证过程中，与动物试验进行对比**，准确率达70%。

2015年某作者文章(审稿)：“**SkinEthicTM**重组皮肤模型对无水乳液的刺激性评估”

---- **SkinEthicTM**购于美国**MatTek**公司。

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# 支持国内企业使用替代方法

目前化妆品动物实验存在的问题：

1. 动物实验质量：行政许可？CNAS认可？AAALAC认证？
2. 非特备案检测机构恶性竞争？
3. 动物实验结果的使用

-----目的：合格？不合格？

-----作用：保证产品安全？提升产品质量？

-----报告内容：过于简单，过程不透明，GLP规范？

4. 安全性测试的看法

-----越便宜越好？

-----只是为了报批或报告？

-----了解产品的安全性？为了质量持续改进？

接受和应用替代方法，你准备好了吗？

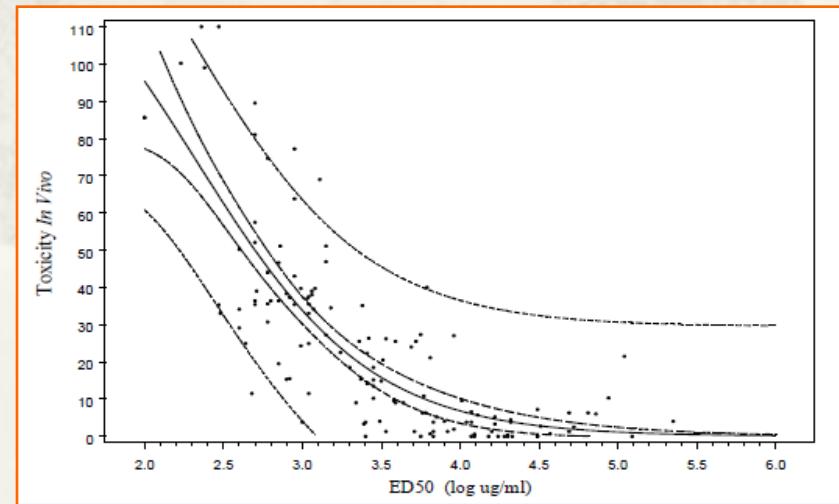
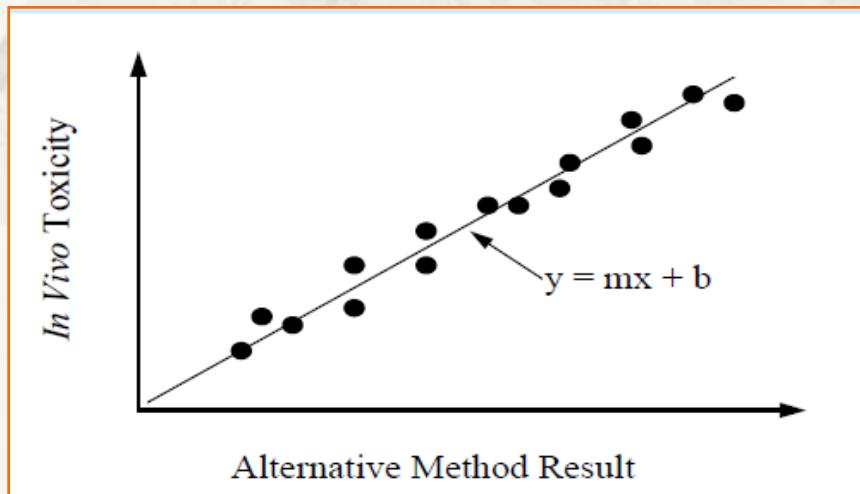
# 附、替代方法的标准化与验证

体外实验≠替代方法

替代方法=体外实验+预测模型

预测模型

体内与体外的相关性：可能很简单，可能很复杂



# 替代方法的标准化与验证

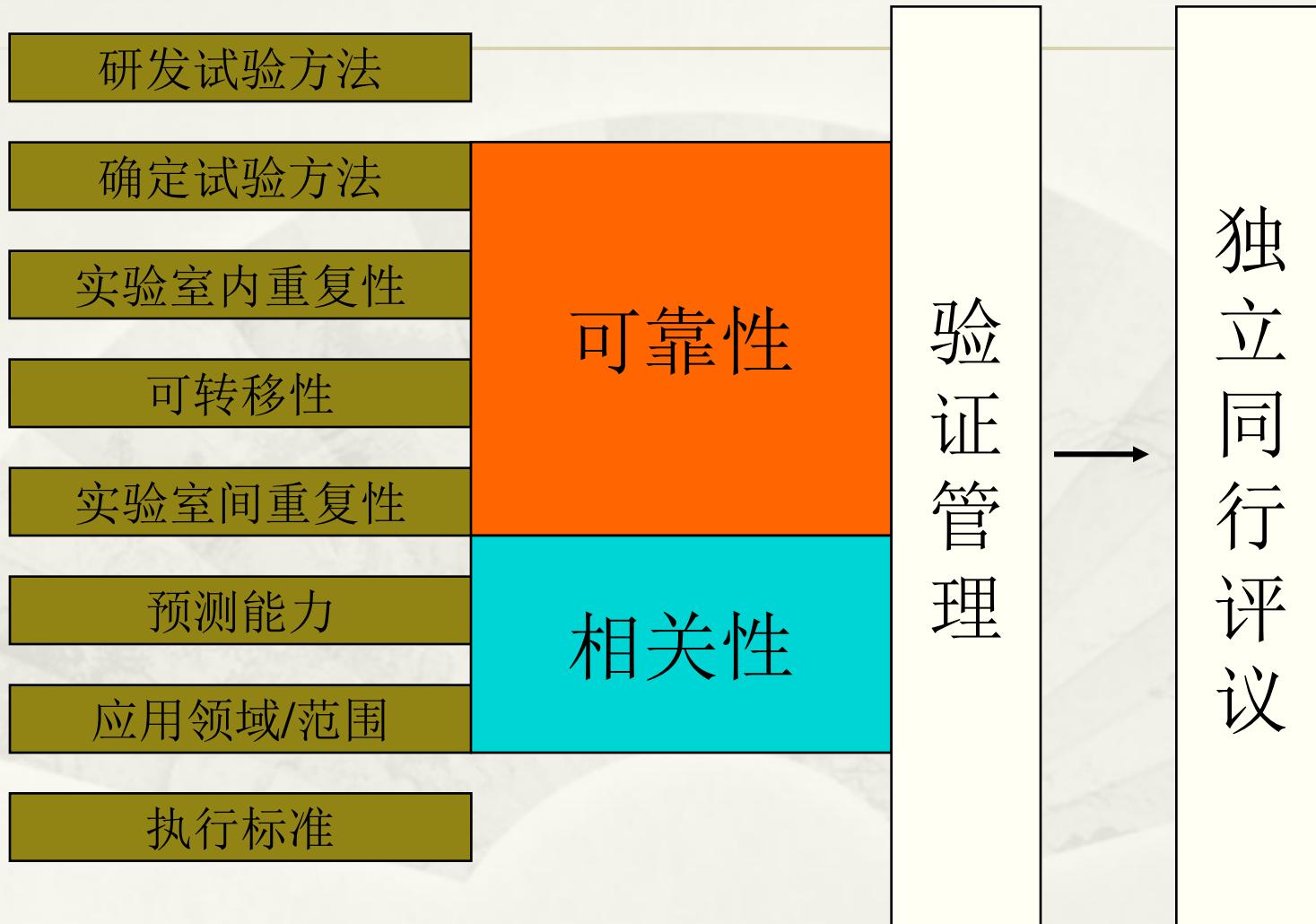
什么是验证Validation?

为了明确的目的，对特定试验、方法、程序或评价的相关性和可靠性建立程序的过程。

为什么要验证？

- 与体内试验不同的系统的评价模型
- 替代技术成为趋势
- 技术革新和毒理学现代需要
- 与动物试验比较
- 说明与人体健康的相关性

# 验证的目的



×为了替代而替代，无健康或环境相关性

# 验证的评价指标

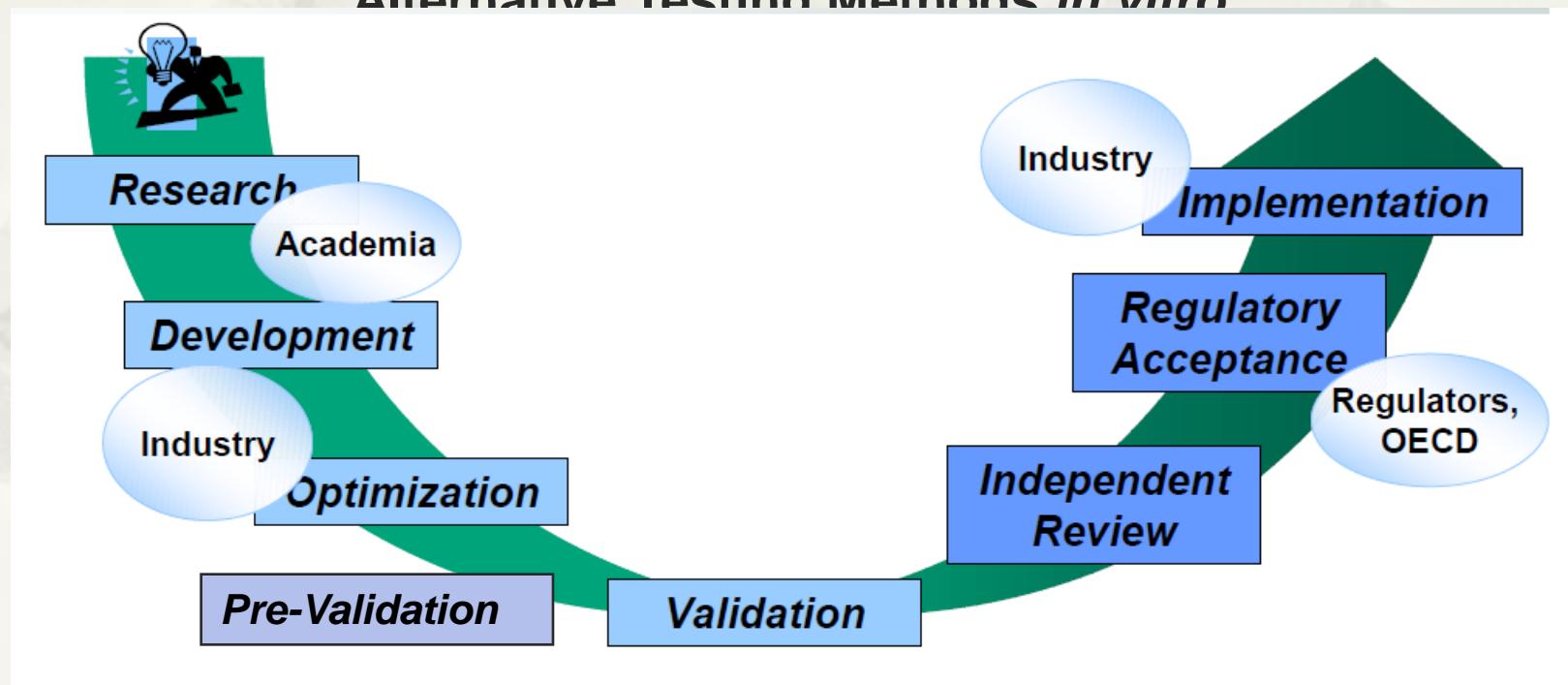
试验结果	参考受试物质		总计
	阳性	阴性	
阳性	a	b	a+b
阴性	c	d	c+d
总计	a+c	b+d	a+b+c+d

再现性Reproducibility, 准确性Accuracy, 假阳性False positive  
假阳性率False positive rate, 假阴性False negative,  
假阴性率False negative rate, 重复性 Repeatability,  
预测性Predictivity, 可靠性Reliability, 敏感性Sensitivity  
特异性Specificity, 混合率 Prevalence, 预测模型Prediction models  
普适性或可转移性Transferability

# Procedure of Validation

验证的标准：化妆品体外替代试验方法  
验证和认可规程（SN/T 3898）

Guideline for the Validation and  
Acceptance of Cosmetics  
*Alternative Testing Methods *in vitro**



# 学术探索与创新

## 1 替代方法共识平台

Chinese Center for Alternatives Research & Evaluation (CCARE)

<http://www.vitrotox.com>

## 2. 学术专著

《实验动物替代方法原理与应用》科学出版社,2006-2010

《3R和仁慈准则》2014

《化妆品科学与评估》6卷本, 2014—2016

《预测毒理学与替代技术指南》, 2010-2015



## 体外实验方法的实操培训



2010~2014



"This is ridiculous. We can't test the products on animals, but should we test them on human beings instead?"  
XENOPOLIS, A NETWORK ON GLOBAL POLICY WITH CONCERN FOR SCIENCE IN POLICY

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## **Testing:** A humane move, or a cosmetic change?

FROM PAGE 1

"It will be a very good thing for domestic manufacturers in China, because it will enable them to sell their products in the U.S. as long as no new testing has been done for their products since March 11, 2003," said Troy Seidle, director of the Research and Toxicological Department with the United States-based Human Health Safety Council.

However, he added, China still has a long way to go before it can match international standards in the use of *in vitro* methods — tests conducted on cell cultures in petri dishes or test tubes — and other alternative methods.

"It is not simply a shift in testing methods from Western to in-kind methods. There is still much more work to be done in the technological aspects and in technological standards," said Cheng Shulin, director of the Toxicology Department with the Technology Center at the Guangdong Entry-Exit Inspection and Quarantine Bureau.

Cheng said it has been an arduous testing road to promotion, as the industry was very conservative and many companies were demanding changes in terms of ground breaking. "When not easily opposed, in certain testing methods, We can only try our best to catch up," he said.

Zhang Quanbin, a senior scientist and program manager with

The Institute for Viro Sciences in the United States, and it could take a long time for Chinese laboratories to change the way they conduct research. "Unluckily, the country's cosmetics industry has undergone rapid growth, yet in terms of testing methods and technology, it lags far behind," says Wang.

According to Zhang, only two Chinese companies currently have the ability to produce 3D cultures — which simulated environments that allow cells to grow and interact with the environment in all three dimensions — for testing, while in the US there is a whole industry that, "After all, the priority is to guarantee the safety of consumers," he said.

**A late start**  
Compared with European countries and the United States, China has had a late start in the development of its micro-methods, and the country's scientists only started researching the problem about a decade ago, according to Cheng, who said very few scientists conducted research into alternative testing in the early part of the century.  
"In recent years, these methods have attracted the attention of a growing number of researchers and users in China, and research is slowly progressing," he said. "But compared with Western countries, there is still a huge gap."

The relaxation of the test requirements for some cosmetic products has raised concerns among Chinese consumers. A number of netizens on *geek*, a social networking site for science enthusiasts, wondered if the phasing out of *in vivo* testing would compromise product safety. "This is ridiculous. We can't just let the

products on animals, but should we let them on human beings instead?" wrote a notitia under the username KaterinaP.

These concerns were dismissed by some European scientists, who said the change won't automatically mean that products will become less safe.

"In the UK, we ceased testing cosmetics on animals in 1988, and have experienced no problems as a consequence," We welcome the suggestion

"The Chinese market is very large and we are happy to share our experience with colleagues here," said Lydia Macleod CBE, head of the Animals in Science Regulation Unit at the United Kingdom's Home Office.

She warned that the new testing methods would require a large amount of investment, which could present challenges for some testing laboratories and cosmetics manufacturers. "There will be some initial set-up costs for Chinese cosmetics companies while they retrain their staff so that

A group of people, including a man and several women, gathered around a table in a laboratory setting. A person in a white lab coat and blue gloves is demonstrating something to the group. The group is looking intently at the demonstration.

A photograph showing a laboratory setting. On the left, a scientist wearing a white lab coat and blue gloves is using a pipette to transfer a yellow liquid from a small white dish into a multi-well plate. The multi-well plate is held by another person whose hands are visible. In the background, another scientist wearing a full-body blue protective suit and mask is standing near a piece of laboratory equipment. The scene illustrates the use of alternative procedures in research.

For more information about the Chinese government's policies on animal testing, visit the website of the Chinese Academy of Agricultural Sciences ([www.caas.net.cn](http://www.caas.net.cn)) or the Chinese Ministry of Science and Technology ([www.most.gov.cn](http://www.most.gov.cn)).  
The Chinese government has issued a series of regulations and guidelines for the use of animals in scientific research, including the "Regulations on the Administration of Animal Experimentation" and the "Guidelines for the Welfare of Animals Used in Scientific Research". These regulations require researchers to follow strict ethical standards and to minimize the suffering of animals used in experiments.

**There is a need for a national coordination mechanism to popularize alternative testing methods**

CHENG SHUAI, DIRECTOR OF THE INSTITUTE OF INNOVATION IN TECHNOLOGY CENTER OF THE CHINA ACADEMY OF FARMING AND RURAL DEVELOPMENT

While this may dictate the amount of time which will be both available and appropriate for the course, it will have an impact on the course's design. Compared with other courses, there are advantages in terms of creating time and space for learning and teaching, and the potential for more time available to humanise the course.

"Human skin is very different from that of animals such as dogs, so it's not appropriate for that kind of experiment to take place," he said.

However, because some alternative methods do not fully reflect the use of live animals, an outright ban on all of its use is not yet feasible, he said.

For example, the *Journal of Cosmetic Dermatology* has proposed a "gradual approach" to animal testing, which would allow companies to use animal testing if they wanted to, but not require it, according to the journal's editor, Dr. Michael J. Karras.

The journal's proposal, which was published online in April, has been widely supported by the cosmetics industry, according to Dr. David C. Gitterman, president of the International Society for Animal Rights.

"The journal's proposal is a good one, and it's something that we can work with," he said. "It's a good starting point, the premise of which is that animal testing in China will require the involvement of scientists, because many cosmetic products are complex and unable to fully comprehend the strengths of live methods and animal testing."

"More research is needed to know

# 验证活动举例



## 3T3 中性红摄 取光毒性验证

EU → In the year 2000 for the first time in vitro toxicity test accepted by the EU into Annex V of Directive 67/548/EEC on the Classification, Packaging and Labelling of Dangerous Substances.

OECD → 2004 worldwide acceptance of the 3T3NRU PT test

## 多中心皮肤刺激性测试验证项目 ----EpiSkin重建表皮模型的应用

### Multi-Center Study of an *in vitro* Alternative Method

--Validation of *in vitro* Skin Irritation Testing Method Using EpiSkin Model

# 谢谢！

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