

化妆品毒理安全性评价技术的革命

上海出入境检验检疫局动植物与食品检验
检疫技术中心

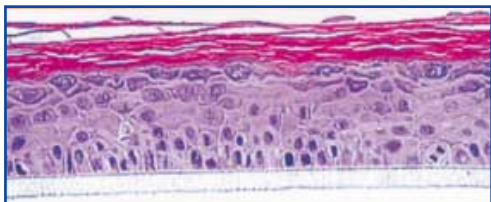
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内容

- 化妆品（药妆）毒理安全性的传统模式
- 动物福利与毒理替代试验
- 替代试验在世界、在中国



传统模式

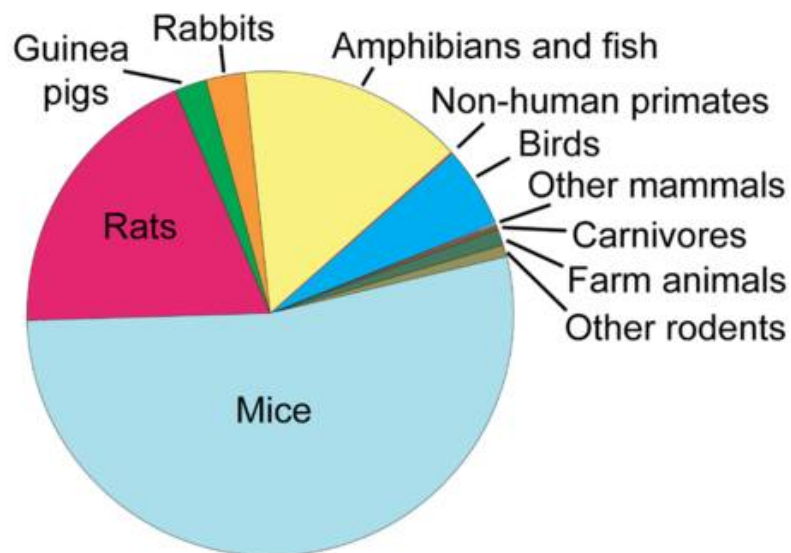
- 《化妆品卫生规范》（2007）第二部分
急性经口，局部毒性，遗传毒性（睾丸细胞），生殖发育毒性，亚慢性毒性，慢性/致癌性-17项。
- 动物试验为主
体外项目：Ames，体外哺乳动物细胞染色体畸变，体外哺乳动物细胞基因突变-3项。



传统模式

- **Animal test**

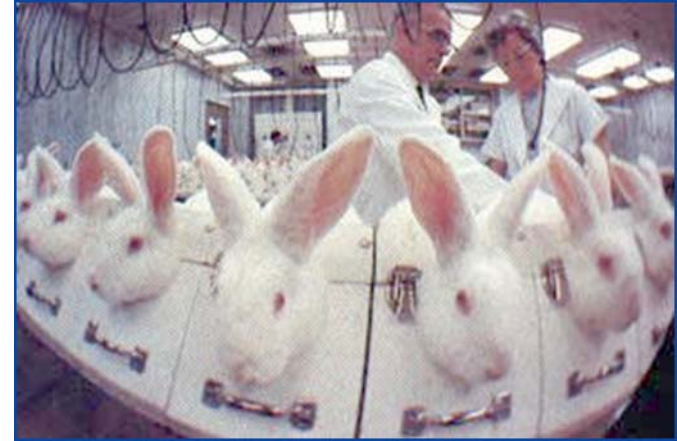
50 to 100 million animals such as rats, mice, rabbits, guinea pigs, birds and other animals are used every year in medical and cosmetic tests.



传统模式

- Two of the most common tests :
Draize test, LD₅₀ tests.

The Draize test is when a substance is dropped directly into an animal eye (normally an albino rabbit) and the results are recorded. The LD₅₀ tests stands for Lethal Dose 50 and it is when a substance is force fed to animals until 50% die.



动物福利与替代试验

- 3R

《The Principles of Humane Experimental Technique》
(1959)



- Russell
- Burch



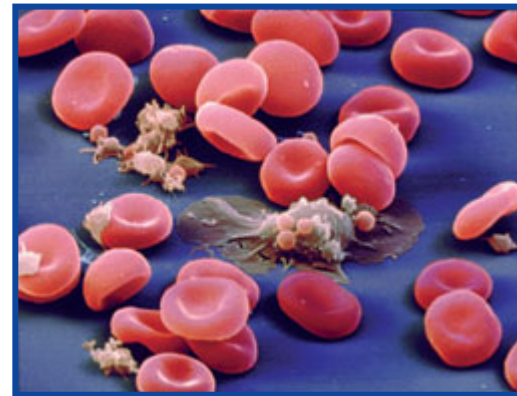
3Rs

- **Reduction** - fewer animals
- **Refinement** - less painful procedures
- **Replacement** - alternative techniques

Absolute replacement

Relative replacement

Partial replacement



3Rs+1

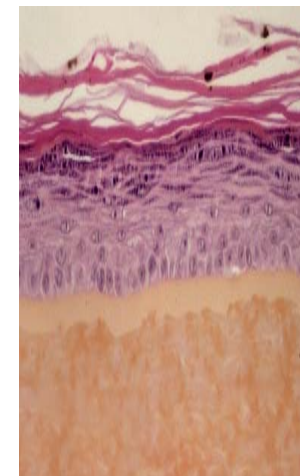
- **Reduction** - fewer animals
- **Refinement** - less painful procedures
- **Replacement** - alternative techniques
- **Responsibility**



动物福利与替代试验



- **Safety testing of chemicals**
 - Local toxicity
 - Genotoxicity
 - Screening tests
- **Quality control and potency testing of vaccines**
- **Production of monoclonal antibodies**



Regulatory Acceptance

- Acute aquatic toxicity
 1. Fish acute toxicity : OECD: Guidance Document No. 126 published in May 2010
- Acute oral toxicity
 1. Acute Toxic Class Method for acute oral toxicity testing : OECD:423
 2. Fixed Dose Procedure for acute oral toxicity testing : OECD:420
 3. Up-and-Down Procedure for acute oral toxicity testing : OECD:425
- Eye irritation
 1. Isolated Chicken Eye (ICE) test method : OECD:438
 2. The Bovine Corneal Opacity and Permeability test methods (BCOP) : OECD:437
- Genotoxicity
 1. In vitro Mammalian Cell Micronucleus Test : OECD:487
- Phototoxicity
 1. 3T3 NRU phototoxicity test Test : OECD:487
- Pyrogenicity
 - Five *In Vitro* Pyrogen Test : [EDQM/European Pharmacopoeia](#)
- Skin absorption
 1. In vitro tests for skin absorption : OECD:428



Regulatory Acceptance

- Skin corrosion

1. EST-1000 method for skin corrosivity testing : OECD:431
2. SkinEthic™ Human Skin Model for skin corrosivity testing : OECD:431
3. CORROSITEX assay for skin corrosivity : OECD:435
4. EpiDerm™ skin corrosivity test : OECD:431
5. EPIKIN™ skin corrosivity test : OECD:431
6. Rat TER skin corrosivity test : OECD:430

- Skin irritation

1. Two *in vitro* skin irritation tests: Modified EpiDerm SIT and SkinEthic™ RHE assay : OECD:439
2. Artificial skin models (EpiSkin®, EpiDerm®) for skin irritation testing : OECD:439

- Skin sensitisation

1. Reduced Local Lymph Node Assay (rLLNA) for skin sensitisation : OECD:429
2. Local Lymph Node Assay for skin sensitisation (LLNA) : OECD:429

- Vaccines: potency and safety testing

4 method.

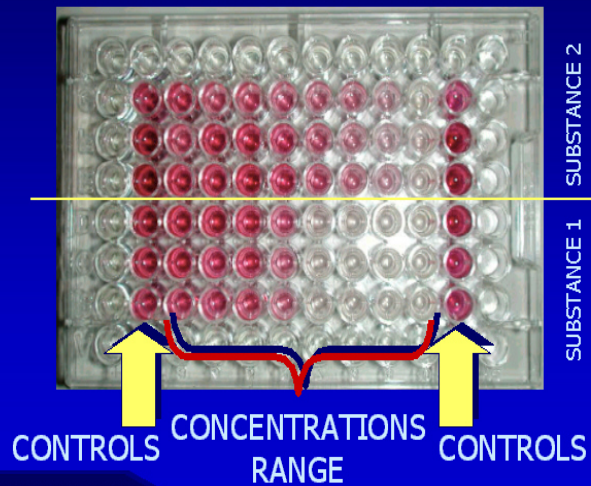


最新被OECD接受的替代方法

- Acute aquatic toxicity (May 2010): Short Guidance on the Threshold Approach for Acute Fish Toxicity
- Genotoxicity (July 2010): *in vitro* micronucleus (MNvit) assay
- Skin irritation (July 2010): EpiSkin™, the modified EpiDerm™ and the SkinEthic™ RhE)
- Skin sensitisation (July 2010): The revised OECD Test Guidelines No. 429 describes the Local Lymph Node Assay (LLNA)
- Eye irritation (September 2009): referring to the Bovine Corneal Opacity and Permeability (BCOP) and on the Isolated Chicken Eye (ICE)

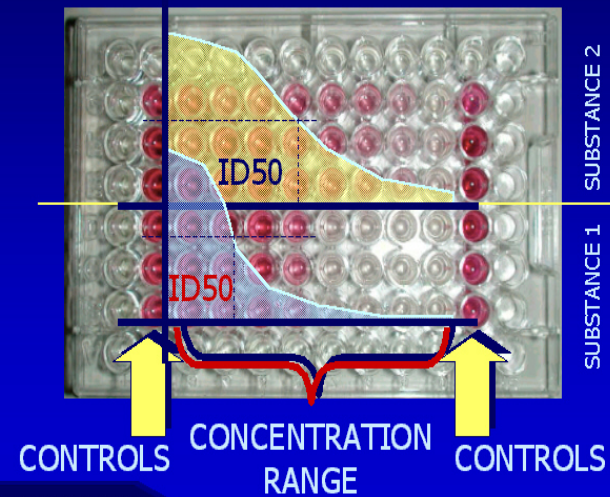


Plate ready to read: 2 substances



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Results: 2 substances

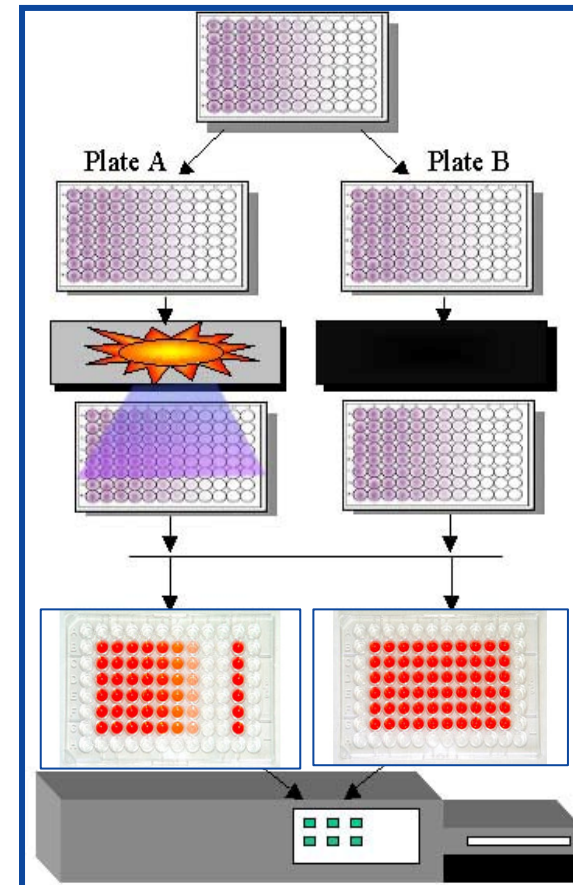


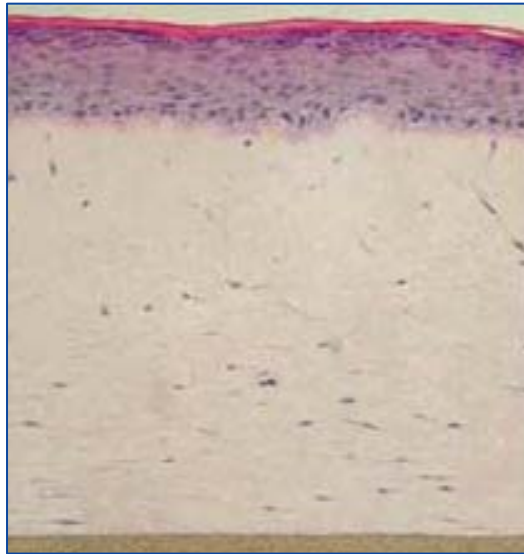
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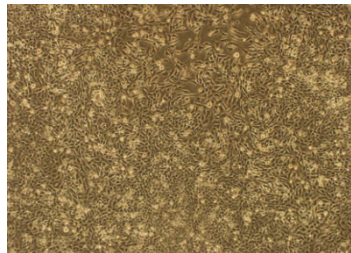
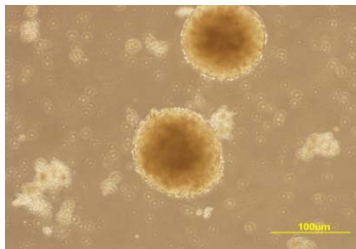
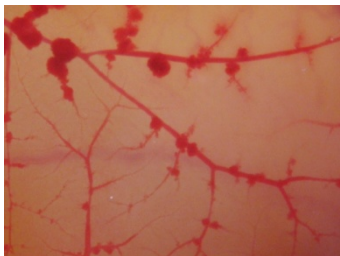
- Mouse fibroblast 3T3 cell line
- Comparison of cytotoxicity
- presence and absence of exposure to a noncytotoxic dose of UVA
- Neutral Red Uptake (NRU) assay





替代试验在世界、在中国

- Directive and Regulation
- Institution
- Research



3R指导下的指令和法规

7th Amendment to the Cosmetics Directive (76/768/EEC)

Testing ban	
Finished cosmetic products	Sep. 11, 2004
Cosmetic ingredients	As soon as alternative methods are validated and adopted, <u>latest Mar. 11, 2009 irrespective of availability of alternative non-animal tests</u>
Marketing ban	
Finished cosmetic products and ingredients	As soon as alternative methods are validated and adopted, latest Mar. 11, 2009 for all human health effects except repeated dose toxicity, reproductive toxicity and toxicokinetics
	Mar. 11, 2013 for all effects irrespective of availability of alternative non-animal tests



欧洲议会和欧盟理事会化妆品法规

(EC) 1223/2009



- Legal document
- Revise and conclude (76/768/EEC)
- Clear the definition of cosmetics
- Nammo

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3R指导下的指令和法规

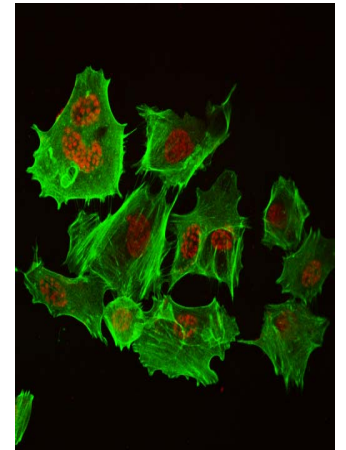
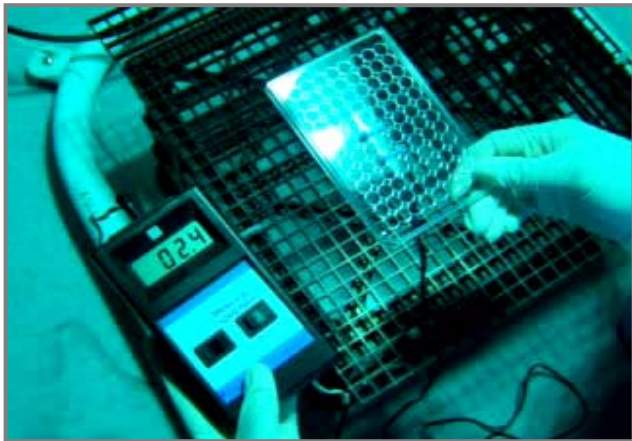
EU Directive 86/609/EEC

- An experiment shall not be performed on an animal, if another scientifically satisfactory method of obtaining the result sought, is reasonably and practicably available.
- The European Commission and the EU Member States must actively encourage and support the development, validation and acceptance of methods which could reduce, refine or replace the use of laboratory animals.



REACH :

Registration, Evaluation, Authorization and
Restriction of Chemicals



验证协调机构

- **ECVAM**, European center for the validation of Alternative method
- **ICCVAM**, the interagency coordinating committee on the validation of alternative methods,
- **JaCVAM**, Japanese Center for the Validation Alternative Methods
- **KoCVAM**, Korean Center for the Validation of Alternative Methods



研究和服务机构

- **Enterprise**

America: CAAT, the John Hopkins center for alternatives to animal experiment, Altweb。

UCCAA, University of California center for animal alternatives...

German: ZEBET, the center for documentation and evaluation of alternative methods to animal experiment, (AnimAlt-ZEBET)。

Netherlands: NCA, Netherlands center alternatives to animal use。

Britian: NC3Rs, The National Centre for the Replacement, Refinement and Reduction of Animals in Research
FRAME, fund for the replacement of animal in medical experiment,
FRAME web



毒理替代在中国

国家标准 National Standard

- Chemical—In vitro 3T3 NRU phototoxicity test method , 化学品体外3T3中性红摄取光毒性试验方 法GB/T 21769-2008
- Chemicals acute oral toxicity-toxic class method, 化学品 急性经口毒性试验 急性毒性分类法GB/T 21757-2008
- Chemical—Test method of skin sensitization-Local lymph node assay, 化学品 皮肤变态反应试验 局部淋巴结方法GB/T 21827-2008
- Acute Oral Toxicity-Fixed Dosed Method for chemicals , 化学品急性毒性试验-固定剂量法
- Acute Oral Toxicity - Up and Down Procedure, 化学品急性毒性试验-上下增减剂量法
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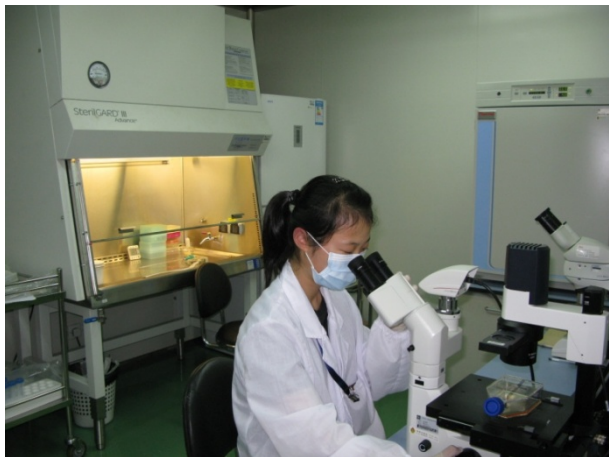
行业标准Industry Standard

- *Good Laboratory Practice for Cosmetics Alternative Testing *In vitro*, 化妆品体外替代试验实验室规范
- *Cosmetics Acute Toxicity of Keratinocyte Cytotoxicity Test , 化妆品急性毒性的角质细胞试验
- *Cosmetics Ocular Irritant and Corrosive HET-CAM Test, 化妆品眼刺激性/腐蚀性的鸡胚绒毛尿囊膜试验
- *Cosmetics Embryotoxicity and Developmental Toxicity of Mice Embryonic Stem Cell Test, 化妆品生殖和发育毒性的小鼠胚胎干细胞试验



行业标准Industry Standard

- 化妆品-SIRC细胞STE眼刺激筛选试验
- 化妆品-细胞培养试验良好操作规范
- 化妆品-生殖毒性：体外全胚胎培养法
- 化妆品-红细胞光毒反应试验
- 化学品-皮肤变态反应LLNA法



We are ready? !

- 中国政府部门承受压力
- 中国国内还没有专门的协调机构和制约法规
- 贸易的需要
- 对企业自律有利于发展
- 药妆产品



Thanks!

