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化妝品用中藥安全性測試（二）

委 託 研 究 報 告

計劃委託機關：中國醫藥學院

計劃主持人：陳甘霖

研究人員：張惠淇、楊惠雯、陳鈺如、胡峻銘、蔡睿盈

執行期間：90 年 09 月 28 日至 90 年 12 月 31 日止

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行政院衛生署中醫藥委員會九十年度委託研究計劃成果報告

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執 行 機 構　　中國醫藥學院

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執行單位　中國醫藥學院

摘 要

化妝品使用於人的皮膚，因此必須對皮膚不產生任何傷害。故中藥化妝品中所使用的中藥材與其他賦型劑一樣，先要瞭解其安全性。

化妝品之安全性試驗包括:毒性試驗、皮膚刺激性試驗、眼睛刺激性試驗、單一劑量口服致死劑量 (LD_{50}) 和致突變毒性等之測定。本計畫用老鼠測定單一劑量口服致死劑量 (LD_{50})。兔子皮膚刺激性試驗和兔子眼睛刺激性試驗依照常用的 Draize 氏法評估計算刺激性的大小。以沙門氏菌測試致突變性 (Ames Test) 研究中藥致的突變性。

本計畫將進行化妝品可用之十五種中藥材之安全性試驗: 龍腦香（冰片）、惡實（牛蒡）、艾草、連翹、梔子花、山楂、牛膝、丹參、冬青葉、水萍、冬瓜子、地膚子、薏仁、百合、黃蓮。除地膚子與黃蓮之 Ames Test 外，其餘均無安全性顧慮。

關鍵詞： 中藥　化妝品　安全性試驗

Abstract

Cosmetics shouldn't have damage in skin when used in human. Chinese herbs, like other excipients, when used in cosmetics must be considered their safety.

The safety tests in cosmetics including: toxicity studies, skin irritation study, eye irritation study, metagenicity study etc. In this study, single dose toxicity was conducted in mice. Degrees of skin and eye irritation studies in rabbits were evaluated by the scoring system of Draize test. The Ames test was applied to the metagenicity study of the Chinese herbs.

Fifteen Chinese herbs were studied in this safety test. They were: *Arctium lappa* L., *Artemisia argyi* Levl. et Vant, *Achyranthes bidentata* Blume, *Benincasa hispida* (Thunb.) Cogn, *Crataegus pinnatifida* Bunge, *Dryobalanops aromatica* Gaertn. f., *Coix lacryma-jobi* L. var. *ma-yuen* (Roman.) Stapf, *Coptis chinensis* Wallich, *Forsythia suspense* (Thunb.) Vahl, *Gardenia jasminoides* Elles, *Ilex chinensis* Sims, *Kochia scoparia* Schrad, *Lilium brownii* F.E.Brown var *colchesteri* Wipls, *Saliva miltiorrhiza* Bunge, and *Spirodela polyrrhiza* Schleid. None of them was considered toxic except the Ames test of *Coptis chinensis* Wallich and *Kochia scoparia* Schrad. They need further comfirm.

前 言

研究和整理傳統醫藥中的美容植物藥，從而研發出天然活性美容化妝品是順應回歸自然、回歸大自然的趨勢。亦為世界各國普遍重視傳統醫學的研究。植物除藥用之外，用於保健美容的植物藥研究和開發迅速。中醫藥的美容，傳統醫藥之最早著作《神農本草經》就收載了。多種可用於美容的植物藥如: 澤瀉“味甘，寒。久服延年輕身，面生光，能行水上”；紫芝“味甘，溫。久服好顏色，輕身不老，延年”；決明子“味咸，平。久服益精光，輕身”；旋花“味甘，溫。去面黑乾黑色，媚好，輕身”；柏實“味甘，平。久服令人潤澤美色，耳目聰明，不飢不老，輕身延年”；白芷“味辛，溫。長肌膚，潤澤，可作面脂”；秦椒“味辛，溫。堅齒發，明目，久服輕身，好顏色，耐老增年”；翹根“味甘，寒。益陰精，令人面悅好，明目，久服輕身耐老”；卷柏“味辛，溫。久服輕身，和顏色”；桃花“味苦，平。令人好顏色”〔梁・陶弘景編. 尚誌鈞， 尚元勝輯校 .本草經集注 (輯較本).北京:人民衛生出版社， 1994:1～551〕。其後， 東晉葛洪《肘後備急方》，梁朝陶弘景的《肘後百一方》，唐 孫思邈《備急千金要方》和《千金翼方》，王燾《外台秘要》均有應用美容植物藥的美容方劑，並沿傳至今。亞洲人女性喜愛美膚、增白、祛斑; 這樣的問題日本、

韓國、泰國、越南等國也非常關心。日本醫藥學家丹波康賴於公元982～984年編撰的《醫心方》30卷，輯錄有中國晉唐方書200餘種，卷4論皮膚病，卷26、27養生等卷均有美容植物藥應用的記載。朝鮮醫官金禮蒙於公元1445年編撰的《醫方類聚》，收輯中國明代以前的醫籍153種，以及一些非醫書文獻之有關內容加以匯編而成，全書共262卷，涉及美容方面的有“頭面”、“毛髮”、“諸湯”、“諸香”、“養性”、“婦人”等卷，包含了許多美容植物藥應用的記載。

印度傳統醫藥中的美容植物藥主要是Ayurveda、Unani、Sida之醫典的記載。印度醫療保健的植物約7500種，其中Ayurveda、Unani、Sida使用的約有1200種中，美容用的植物藥不少。如：用於治療面部斑疹疾患，包括痤瘡、粉刺、面皰、水泡、雀斑、丘疹、疙瘩、粉刺等。可用於醫學美容和治療損容性皮膚病的植物藥有：

Achyranthes 牛膝屬, Allium 蔥屬, Amorphophallus 魔芋屬, Anacardium 腰果屬, Anthocephalus 團花屬, Artemisia 蒿屬, Begonia 秋海棠屬, Bombax 木棉屬, Buchanania 山木姜子屬, Butea 紫鉚屬, Capparis 槌果藤屬, Casearia 嘉賜樹屬, Cassia 決明屬, Cissampelos 錫生藤屬, Citrus 柑屬, Clematis 鐵線蓮屬, Commelina 鴨跖草屬, Curcuma 薑黃屬, Dalbergia 檀屬, Datura 曼陀羅屬, Dendrophthoe 五蕊寄生屬, Desmodium 山螞蝗屬, Diospyros 柿屬,

Elephantopus 地膽草屬, Erythrina 刺桐屬, Euphorbia 大戟屬, Ficus 榕屬, Gardenia 梔子屬, Grewia 扁擔杆屬, Hedyotis 耳草屬, Hordeum 大麥屬, Ichnocarpus 腰骨藤屬, Iris 鳶尾屬, Jurinea 苓菊屬, Lannea 厚皮樹屬, Leptodermis 野丁香屬, Limnopila 石龍尾屬, Lyonia 南燭屬, Mallotus 野桐屬, Mirabilis 紫茉莉屬, Mucuna 黎豆屬, Murraya 九里香屬, Nelsonia 瘤子草屬, Nerium 夾竹桃屬, Opuntia 仙人掌屬, Oroxylum 千張紙屬, Phyllanthus 葉下珠屬, Premna 豆腐柴屬, Psidium 番石榴屬, Punica 安石榴屬, Raphanus 蘿卜屬, Sesbania 田菁屬, Smilax 菝契屬, Solanum 茄屬, Sterculia 苹婆屬, Syzygium 蒲桃屬, Taraxacum 蒲公英屬, Tridax 羽芒菊屬, Vernonia 斑鳩菊屬, Vitex 牡荊屬的多種藥用植物〔S. K . Jain: Dictionary of Indian Folk Medicine and Ethnobotany. New Deihi (India); Deep Publications, 1991, p.311〕。

南美洲民間的面部皮膚問題的有粉刺、丘疹(pimples)、紅粉刺(red pimples) 、白粉刺(white pimple)、白斑(white spots)、搔痒的丘疹(itching pimples)等。馬雅族統醫藥中的美容治療植物藥，如:以菊科植物 *Caleaurticifolia Mill* sp. var. yucatanensis Wussow, Urb. and Sullivan 的葉子用於治療 red pimples, 以柿科柿屬植物 *Diospyros anisandra* Blake 的葉子治療 itching pimples; 以唇形科

羅勒屬植物 *Ocimum micranthum* Willd.的葉子、氣生部分 pimples, white spots; 以唇形科鼠尾草屬植物 *Salviam icranthum* Vahl 的葉子、氣生部分治療 scabies, pimples; 以茜草科波利亞草屬植物 Borreria verticillata (L .) G . Mey.的氣生部分治療 small, white pimple; 以蘭科植物 *Catasetum integerrimum* Hook.的葉子治療 big pimples; 以大戟科巴豆屬植物 *Croton peraeruginosus* Croizat 的漿汁、葉子治療 pimples; 以茜草科植物 *Hamelia patens* Oacq. 的葉子治療 red pimples; 以苦木科植物 *Alvaradoa amorphoides* Liebm .的葉子治療 itching pimples; 以豆科植物 *Dalea carthagenensis* var. barbata (Oerst.) Barneby 的葉子治療 pimples; 以桃金娘科番石榴屬 *Psidium guajava* L .的葉子治療 pimples; 以豆科植物 *Senna villosa* (Mill.) Irwin and Barneby 的葉子治療 hard, little pimples 等〔A. Anita; O. Sticher, and M. Heinrich: Midical Ethnobotany of the Yucatec Maya. Economic Botany, 1999; 53 (2):144～160〕。

化妝品是為:清潔、美化身體，增加魅力，改變容貌，或者為保護皮膚和頭發，而塗沫、撒布在身體上的對人體作用緩和的製品稱為美容化妝品〔(日)光井武夫主編，新化妝品學.〕。由於美容化妝品是日常生活中每天或長期連續被使用的物質，因此美容化妝品應該是安

全的，無毒副作用的。傳統中醫藥，幾千年的應用中已積累了許多藥效記錄和經驗，對它們的用量、安全性和副作用也有許多驗証，這是合成化合物無法比的。傳統醫藥美容植物藥中所含有的天然活性物，具多重效能，藥效持久穩定，作用溫和，適用廣，副作用無或很小。美容化妝品使用於人的皮膚，因此與其他賦型劑一樣，中藥化妝品的安全性和有用性，是化妝品研製和開發先要瞭解的目標。中藥材必須對皮膚不產生任何傷害。

化妝品之安全性試驗包括:毒性試驗、皮膚刺激性試驗、眼睛刺激性試驗、和致突變毒性等之測定。測試的模式有很多方法，本計畫毒性試驗係測定老鼠口服單一致死劑量 (LD_{50})。刺激性試驗用兔子皮膚刺激性試驗和兔子眼睛刺激性試驗表示之。依照常用的 Draize 氏法評估計算刺激性的大小。以沙門氏菌測試致突變性 (Ames Test) 研究中藥致的突變性。本計畫進行十五種中藥材之安全性試驗。

中藥使用在化妝品，由來已久，國科會的生物技術計畫中，有結合中藥和醫學的研究計畫，如果能擴展中藥應用到化妝品，將能有效地增加中藥的經濟價值。

實驗材料與方法

材料--

選用化妝品可用之實驗中藥材，冰片、牛蒡、艾草、連翹、梔子花、山楂、牛膝、丹參、冬青葉、水萍、冬瓜子、地膚子、薏仁、百合、黃連等十五種購自台中市聯合中西藥局及太平鮮花店，並經本校邱年永老師鑑定。基原如下：

牛蒡 菊科植物牛蒡 *Arctium lappa* L. 的根

艾草 菊科艾 *Artemisia argyi* Levl. et Vant 的乾燥葉

牛膝 莧科牛膝 *Achyranthes bidentata* Blume 之乾燥根

冬瓜子 葫蘆科冬瓜 *Benincasa hispida* (Thunb.) Cogn 的種子

山楂 薔薇科山楂 *Crataegus pinnatifida* Bunge 的果實

冰片 龍腦香科龍腦香 *Dryobalanops aromatica* Gaertn. f. 樹脂的加工品

薏仁 禾本科薏苡 *Coix lacryma-jobi* L. var. *ma-yuen* (Roman.) Stapf 的種仁

黃連 毛茛科黃連 *Coptis chinensis* Wallich 的乾燥根

乾燥根連翹 木犀科連翹 *Forsythia suspense* (Thunb.) Vahl 的果實

梔子花 茜草科梔子 *Gardenia jasminoides* Elles 之成熟花朵

冬青葉 冬青科植物冬青 *Ilex chinensis* Sims 的葉

地膚子 藜科地膚 *Kochia scoparia* Schrad 的乾燥種子

百合 百合科百合 *Lilium brownii* F.E.Brown var *colchesteri* Wipls 的

丹參 唇形科丹參 *Saliva miltiorrhiza* Bunge 之乾燥根

水萍 浮萍科大萍 *Spirodela polyrrhiza* Schleid 的乾燥全草

設備與儀器--

冷凍乾燥機 Flexi-Dry™ μP, FD-3-85A-MP, FTS SYSTEMS, USA

減壓濃縮機 BUCHLER INSTRUMENTS, USA

烘箱 進信公司， 台北

水浴鍋 EYELA, SB-35, RIKAKIKAI, TOKYO

實驗方法--

中藥材水溶液萃取液之製備方法：

已磨碎之十五種中藥材，各秤取 20g，加入四倍重量之二次水，浸濕後，於 95℃的水浴鍋煎煮 1.5 小時後，趁熱過濾去渣，得第一次濾液。將過濾出之藥渣加入適量體積之二次水，以前述步驟再煎煮 1 小時，合併濾液並將總體積調整為 80 ml。每種藥材萃取液分兩等份，一份將濾液以冷凍乾燥濃縮至乾，稱重後於-30℃下保存，作為計算含量及毒性試驗之 LD_{50} 使用；另一份濾液放冷後置於-30℃冷凍

櫃備用。

50%酒精溶液萃取液製備方法：

已磨碎之十五種中藥材，各秤取 20g，加入四倍重量之二次水，浸濕後，於 80-85℃的水浴鍋煎煮 1.5 小時後，趁熱過濾去渣，得第一次濾液。將過濾出之藥渣加入適量體積之 50%酒精溶液，以前述步驟再煎煮 1 小時，合併濾液並將總體積調整為 80 ml。每種藥材萃取液分兩等份，一份將濾液先減壓濃縮（50-60℃）至 15-20 ml，再以冷凍乾燥濃縮至乾，稱重後於-30℃下保存，作為計算含量及毒性試驗之 LD_{50} 使用；另一份濾液放冷後置於-30℃冷凍櫃備用。

50%酒精萃取液中酒精之去除

50%酒精萃取溶置於 60℃之烘箱中，0.5 小時後，觀察其體積減少一半以上。調整其體積為原體積之一半。

單一劑量毒性試驗--

一、實驗動物

使用購自國科會之 ICR 系小鼠，體重 18-22g，飼養於中國醫藥學院動物中心，動物室之室溫約為 17℃，提供適量的墊料、飼料及飲水，令其適應環境，並且觀察實驗小鼠之健康狀況。

二、劑量範圍

分別以各藥材之 50%酒精溶液萃取物及水萃取物進行單一劑量毒性試驗。投予之最高限界劑量為: 10g 藥材之萃取物 / kg 小鼠體重。

三、實驗方法：

劑量組各使用 ICR 系小鼠 10 隻 (5 雄、5 雌)。投藥前禁食 24 小時，量取一定之實驗劑量，以胃管給藥後，每天觀察二次以上，以確定 ICR 系小鼠之健康情形。並紀錄 72 小時內，可使實驗動物一半死亡之劑量。

皮膚刺激性試驗--

一、實驗動物

使用體重約 2-3 公斤的紐西蘭白兔進行試驗，飼養於中國醫藥學院動物中心，動物室之室溫約為 17℃，提供適量的飼料及飲水，令其適應環境並且觀察紐西蘭白兔之健康狀況；紐西蘭白兔實驗組每組 3 隻（雄性與/或雌性）。

二、劑量範圍

試驗物質為 0.5 ml，各藥材之濃度將以量表表示之。

三、試驗步驟

1. 測試前一天，剃除動物背部毛髮，檢查皮表是否完整，盡量避免傷害皮膚，若皮表有刮痕或皮膚病，則不予使用。
2. 測試當天，將紐西蘭白兔背部分為六個區域，試驗物質塗抹於動物背部皮表 (約 6cm^2)，並覆蓋上透氣繃帶。選擇其中一塊區域大小相同的皮表，不給予任何試驗物質作為空白對照組。
3. 紐西蘭白兔經試驗物質處理(約 4 小時)後，以二次水清除皮表之試驗物質，觀察並記錄塗抹部位的反應。

三、皮膚刺激性評估

經試驗物質處理後的第 1、24、48 及 72 小時，以皮膚刺激性計分系統[1]（Draize 法）評估，觀察及記錄敷藥部位的皮膚反應，包括所產生的紅斑、浮腫情形、刺激作用、腐蝕作用與恢復情形的程度與性質及其他毒性作用。

ACUTE DERMAL IRRITATION / CORROSION.

TABLE：GRADING OF SKIN REACTION

(摘自 OECD (1992). Guideline for Testing of Chemicals No. 404: Acute Dermal Irritation/Corrosion).

Erythema and Eschar Formation

No erythema 0

Very slight erythema (barely perceptible) 1
Well defined erythema 2
Moderate to severe erythema 3
Severe erythema (beet redness) to eschar formation
preventing grading of erythema 4

＊Maximum possible：4

Oedema Formation

No oedema 0
Very slight oedema (barely perceptible) 1
Slight oedema (edges of area well defined by definite rasing) 2
Moderate oedema (raised approximately 1 mm) 3
Severe oedema (raised more than 1 mm and extending beyond
area of exposure) 4

＊Maximum possible： 4

眼睛刺激性試驗--

一、實驗動物

同於皮膚刺激性試驗，使用體重約 2-3 公斤的紐西蘭白兔進行試驗，飼養於中國醫藥學院動物中心，動物室之室溫約為 17℃，提供適量的飼料及飲水，令其適應環境並且觀察紐西蘭白兔之健康狀況；紐西蘭白兔實驗組每組 3 隻（雄性與/或雌性）。

二、劑量範圍

0.1 ml 之除去酒精後之酒精萃取物質。

三、試驗步驟

1. 拉開兔子的下眼瞼，將試驗物質置入紐西蘭白兔的結膜囊中，而另一隻眼睛不作任何處理作為空白對照組。
2. 經試驗物質處理後，觀察並記錄眼睛刺激反應。
3. 若試驗需要，試驗動物眼睛經試驗物質處理 24 小時後可以水清洗眼睛，但須注意清水的體積與流速不會傷害動物之眼睛。

四、眼睛刺激性評估

1. 動物的眼睛經試驗物質處理後第 1、24、48 與 72 小時，以眼睛刺激性計分系統[3]（Draize 法）評估，觀察及記錄眼睛刺激反應，包括檢查眼角膜 (cornea)、虹膜 (iris)、及結膜 (conjunctiva)。
2. 若在 72 小時後眼睛沒有呈現明顯刺激反應，則可終止此試驗。
3. 若試驗物質會引起持久的角膜或其他眼睛刺激現象，須持續觀察及記錄眼睛刺激性反應至第 21 天止，以評估該眼睛傷害性為可逆性或非可逆性。
4. 除結膜、虹膜、及角膜外，其他眼睛組織若出現損傷，都應加以記錄。

TABLE：GRADES FOR OCULAR LESIONS

CORNEA

Opacity : degree of density
(area most dense taken for reading)
No ulceration or opacity 0

Scattered or diffuse areas of opacity

(other than slight dulling of normal luster),

details of iris clearly visible 1

Easily discernible translucent area,

Details of iris slightly obscured 2

Nacrous area, no details of iris visible,

Size of pupil barely discernible 3

Opaque cornea, iris not discernible through the opacity 4

IRIS

Normal 0

Markedly deepened rugae, congestion, swelling,

Moderate circumcorneal hyperaemia, or injection,

Any of these or combination of any thereof.

Iris still reacting to light (sluggish reaction is positive) 1

No reaction to light, haemorrhage, gross destruction

(any or all of these) 2

CONJUNCTIVAE

Redness (refers to palpebral and bulbar conjunctivae, Cornea and iris)

Blood vessels normal 0

Some blood vessels definitely hyperaemic (injected) 1

Diffuse, crimson colour, individual vessels not easily discernible . 2
Diffuse beefy red 3

Chemosis：lids and/or nictating membranes

No swelling 0
Any swelling above normal (includes nictating membranes) 1
Obvious swelling with partial eversion of lids 2
Swelling with lids about half closed 3
Swelling with lids more than half closed 4

微生物致突變性試驗--

一般採用直接平板混合法（direct plate incorporation method）

一、實驗菌種：

一般採用 *Salmonella typhimurium* TA 98 及 *Salmonella typhimurium* TA 100.

二、實驗材料之配置方法，一般為：

1. 大白鼠肝臟微粒體活化酵素 S9 懸浮液之製備方法

大白鼠腹腔注射 Aroclor 1254（500mg/kg），五天後取其肝臟，加入 0.5M KCl 緩衝液以均質機均勻磨碎，以 9000xg 離心 10 分鐘後，取上層液即為 S9 懸浮液。

2. 上層培養基（Top agar）2 ml

0.6% Difco agar + 0.5%NaCl 滅菌，每 100 ml 加入 10 ml 無菌之 0.5mM biotin 及 0.5mM histidine。

3. 底層培養基

1.5% Difco agar + 2% Vogel-Bonnen medium E+2% Glucose

4. S9 混合液之配置

每 1 ml S9 混和液中含有 0.1 ml S9 懸浮液，8 μM $MgCl_2$，23 μM KCl，5 μM Glucose-6-phosphate，4 μM NADP 及 100 μM Sodium phosphate，pH 7.4

三、劑量範圍

試驗物質為 0.1 ml。

四、試驗步驟

1. 將保存於-70℃之菌種接種到肉湯培養基（nutrient broth）中，於 37℃下振搖，14-15 小時後細菌數可達 10 cells / ml。
2. 將 2 ml 上層培養基於 45℃下，依序加入 0.1 ml 測試樣品，0.1 ml TA 98 或 TA 100 菌種懸浮液，視需要加入 0.5 ml 含老鼠肝臟微粒體活化酵素 S9 混合液（microsomal S9 mix）當代謝性活化劑，均勻搖盪後傾倒於底層培養基平板上。
3. 於 37℃培養箱中培養 48 小時，然後計算 his 逆突變（his＋

revertant）菌落數。

4. TA 98 菌株之自然突變數為 18-25（不含 S9）及 30-45（含 S9）；而 TA100 為 90-100（不含 S9）及 100-160（含 S9）。若被測之樣品引起逆突變菌落數超過上述自然突變數菌落二倍以上，即視為該樣品具有致突變能力。

5. 陽性對照組於 TA 98 使用 2-aminofluorene（含 S9）與 picrolonic acid（不含 S9）；而於 TA 100 使用 2-aminofluorene（含 S9）與 4-nitorquinoline-N-oxide（不含 S9）。

結果及討論

中藥材之水溶液及50%酒精溶液其萃取物重量與萃取率的換算表如下:

表一中藥材(十克)之水溶液及50%酒精溶液之萃取率

中藥材名稱	水溶液 40 ml		50%酒精溶液 40 ml	
	萃取物重(g)	萃取率(%)	萃取物重(g)	萃取率(%)
冰　片	0.2818	2.818	0.3529	3.529
牛　蒡	4.5153	45.153	5.082	50.82
艾　草	3.103	31.03	3.5723	35.723
連　翹	2.0436	20.436	1.2656	12.656
梔子花	0.7183	7.183	1.6095	16.095
山　楂	5.0517	50.517	3.7859	37.859
牛　膝	4.8602	48.602	7.5424	75.424
丹　參	4.1555	41.555	3.9039	39.039
冬青葉	2.0699	20.699	1.6444	16.444
水　萍	0.7622	7.622	0.4332	4.332
冬瓜子	0.5228	5.228	0.5511	5.511
地膚子	1.7178	17.178	0.6012	6.012
薏　仁	0.0687	0.687	0.5089	5.089
百　合	1.3083	13.083	1.0617	10.617
黃　連	1.0089	10.089	2.5029	25.029

表二　十五種中藥材之水溶液及50%酒精溶液其萃取體積與萃取物重量(mg)換算表

中藥材名稱	水溶液萃取液		去酒精之50%酒精萃取液	
	0.1 ml	0.5 ml	0.1 ml	0.5 ml
冰　片	0.7045 mg	3.5225 mg	1.7645 mg	8.8225 mg
牛　蒡	11.28825	56.44125	25.41	127.05
艾　草	7.7575	38.7875	17.8615	89.3075
連　翹	5.109	25.545	6.328	31.64
梔子花	1.79575	8.97875	8.0475	40.2375
山　楂	12.62925	63.14625	18.9295	94.6475
牛　膝	12.1505	60.7525	37.712	188.56
丹　參	10.38875	51.94375	19.5195	97.5975
冬青葉	5.17475	25.87375	8.222	41.11
水　萍	1.9055	9.5275	2.166	10.83
冬瓜子	1.307	6.535	2.7555	13.7775
地膚子	4.2945	21.4725	3.006	15.03
薏　仁	0.17175	0.85875	2.5445	12.7225
百　合	3.27075	16.35375	5.3085	26.5425
黃　連	2.52225	12.61125	12.5145	62.5725

50%酒精萃取溶置於60℃之烘箱中，0.5小時後，觀察其體積減少一半以上。調整其體積為原體積之一半。此時酒精之理論含量值為1.0%以下。

單一劑量毒性其結果如表三及表四所示。因試驗物質之毒性很低，故投予量採最高限界劑量[10g 藥材之萃取物 / kg 體重] 投予。依中醫藥典籍記載，試驗物質之毒性很低，故投予量採最高限界劑量[10g 藥材之萃取物 / kg 體重]投予。實驗結果顯示小白鼠全部存活，致死率為零，活動情形與未給藥之對照組無異；十五種中藥材之水及50%酒精萃取物其 LD_{50} 均大於[10g 藥材之萃取物 / kg 體重]。依化妝品的觀點應可視為無毒性。

皮膚刺激性評估其結果如表五及表六所示。皮膚刺激性測試結果顯示，十五種中藥材之水及50%酒精萃取液，均塗抹0.5 ml / 6cm^2 於背部皮膚，處理後的第1、24、48及72小時，並沒有產生紅斑、浮腫等情形，表示此十五種化妝品用的中藥材並無任何的皮膚刺激作用。其外用的安全性是被證實的。

其眼睛刺激性觀察及評分結果如表七至表十所示。將十五種中藥材之水及50%酒精萃取液，於結膜囊給予0.1 ml，使藥物吸收完全，

結果顯示只有冰片之水及50%酒精萃取液在給藥後二十秒有閉眼的情形，隨即睜開。其餘十四種中藥萃取液，均在藥物完全吸收後恢復正常。在處理後的第1、24、48與72小時，並沒有產生分泌物；以Draize法觀察評估，亦無發紅、腫脹或潰爛腐蝕之情形。表示此十五種化妝品用的中藥材並無任何的眼睛刺激作用，可安全的使用於眼用或外用的化妝品、保養品上。

在歐美國研發化妝品的趨勢為 (1) 美容植物藥，天然活性美容化妝品的研發 (2) 提倡用非動物的試驗方法來評估化妝品的安全性，以大量減少動物的屠殺 (3)儘量使用過去動物資料和安全性的資料，節省研發時間人力與金錢。本計畫使用了中醫藥委員會研究計畫報告，編號DOH81-CM-064、DOH82-CM-062、DOH83-CM-018、DOH84-CM-003、DOH-CD21及資料(Ames, 1975; Yin, 1991; Lee, 1988; Mortelmans, 2000)顯示，除黃連、地膚子之外其他中藥材對*Salmonella typhimurium* TA 98及TA 100不具致突變能力。

在歐美國家講究生存權，尊重動物的生命，因此，安全性試驗以大量用非動物的試驗方法來進行。Draize的眼睛，皮膚刺激試驗可能可以用體外試驗來替代。美國大化妝品公司如 P&G及 Bristol Myers Squibb 減少了幾乎90%的動物測試，更多其他公司也照樣。Colgate

Palmolive公司說，現使用的試驗98 %，可於非動物的試驗方法下完成。美國食品及藥物管理局，動物照顧和使用辦公室主任(Animal care and use office) Neil Wilcox , D.V.M. 說，已經證實大多數安全使用老舊可靠成分，基於過去動物資料和安全使用的歷史，最終產品可在人身上試驗。化妝品的Draize試驗，已有其他選擇。

但是，如果美國化妝品公司想要使用新成分時，又如何? 不像藥物上市申請一樣，化妝品不需要美國食品及藥物管理局上市前的批准。然而，如果在銷售以後，出現安全問題時，美國食品及藥物管理局將採取行動，要製造商提供產品的安全資料。若因為，還沒有足夠的資訊，證實新成分用在人體是安全，則食品及藥物管理局，僅接受動物的安全性試驗 (請參考附件一、二)。

表三　以 50%酒精溶液萃取物進行之單一劑量毒性試驗結果

藥　材	死亡率	LD_{50}* (g/kg)
冰　片	0	>10
牛　蒡	0	>10
艾　草	0	>10
連　翹	0	>10
梔子花	0	>10
山　楂	0	>10
牛　膝	0	>10
丹　參	0	>10
冬青葉	0	>10
水　萍	0	>10
冬瓜子	0	>10
地膚子	0	>10
薏　仁	0	>10
百　合	0	>10
黃　連	0	>10

*劑量為 10g 藥材之萃取物/kg 動物體重

表四. 水溶液萃取物進行之單一劑量毒性試驗結果

藥　材	死亡率	LD_{50}* (g/kg)
冰　片	0	>10
牛　蒡	0	>10
艾　草	0	>10
連　翹	0	>10
梔子花	0	>10
山　楂	0	>10
牛　膝	0	>10
丹　參	0	>10
冬青葉	0	>10
水　萍	0	>10
冬瓜子	0	>10
地膚子	0	>10
薏　仁	0	>10
百　合	0	>10
黃　連	0	>10

表五　皮膚刺激性試驗-Draize 評分（50%酒精溶液萃取物）

藥　材	劑量 / 時間	0.5 ml	藥材	劑量 / 時間	0.5 ml
冰　片	1hr	0	冬青葉	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
牛　蒡	1hr	0	水　萍	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
艾　草	1hr	0	冬瓜子	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
連　翹	1hr	0	地膚子	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
梔子花	1hr	0	薏　仁	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
山　楂	1hr	0	百　合	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
牛　膝	1hr	0	黃　連	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
丹　參	1hr	0			
	24hr	0			
	48hr	0			
	72hr	0			

表六 皮膚刺激性試驗-Draize 評分（水溶液萃取物）

藥材	劑量 / 時間	0.5 ml	藥材	劑量 / 時間	0.5 ml
冰片	1hr	0	冬青葉	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
牛蒡	1hr	0	水萍	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
艾草	1hr	0	冬瓜子	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
連翹	1hr	0	地膚子	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
梔子花	1hr	0	薏仁	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
山楂	1hr	0	百合	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
牛膝	1hr	0	黃連	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
丹參	1hr	0			
	24hr	0			
	48hr	0			
	72hr	0			

表七　眼睛刺激性試驗觀察紀錄（50%酒精萃取物；劑量：0.1 ml）

時間 / 藥材	1hr	24hr	48hr	72hr
冰　片	全劑量點完、藥物吸收完全後，閉眼約 15 秒後睜開。	正常、與對照組無差異	正常	正常
牛　蒡	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
艾　草	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
連　翹	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
梔子花	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
山　楂	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
牛　膝	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
丹　參	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
冬青葉	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
水　萍	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
冬瓜子	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
地膚子	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
薏　仁	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
百　合	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
黃　連	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常

表八　眼睛刺激性試驗觀察紀錄（水溶液萃取液；劑量：0.1 ml）

時間 / 藥材	1hr	24hr	48hr	72hr
冰　片	全劑量點完、藥物吸收完全後，閉眼約 10 秒後睜開。	正常、與對照組無差異	正常	正常
牛　蒡	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
艾　草	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
連　翹	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
梔子花	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
山　楂	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
牛　膝	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
丹　參	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
冬青葉	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
水　萍	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
冬瓜子	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
地膚子	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
薏　仁	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
百　合	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常
黃　連	全劑量點完、藥物吸收完全後，眼睛馬上睜開。	正常、與對照組無差異	正常	正常

表九　眼睛刺激性評估-Draize 評分（50%酒精溶液萃取物）

藥材	劑量 / 時間	0.1 ml	藥材	劑量 / 時間	0.1 ml
冰片	1hr	0	冬青葉	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
牛蒡	1hr	0	水萍	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
艾草	1hr	0	冬瓜子	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
連翹	1hr	0	地膚子	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
梔子花	1hr	0	薏仁	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
山楂	1hr	0	百合	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
牛膝	1hr	0	黃連	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
丹參	1hr	0			
	24hr	0			
	48hr	0			
	72hr	0			

表十　眼睛刺激性試驗-Draize 評分（水溶液萃取物）

藥　材	劑量 / 時間	0.1 ml	藥材	劑量 / 時間	0.1 ml
冰　片	1hr	0	冬青葉	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
牛　蒡	1hr	0	水　萍	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
艾　草	1hr	0	冬瓜子	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
連　翹	1hr	0	地膚子	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
梔子花	1hr	0	薏　仁	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
山　楂	1hr	0	百　合	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
牛　膝	1hr	0	黃　連	1hr	0
	24hr	0		24hr	0
	48hr	0		48hr	0
	72hr	0		72hr	0
丹　參	1hr	0			
	24hr	0			
	48hr	0			
	72hr	0			

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The Draize Test

Cosmetic Testing Declines As Search Continues for Alternatives

The Draize eye and skin irritancy tests remain useful targets for animal rights groups, even while U.S. cosmetic companies have significantly reduced testing by switching to non-animal in vitro alternatives wherever possible. Industry giants Proctor & Gamble and Bristol-Myers Squibb have reduced by almost 90 percent or more their reliance on live animal testing for cosmetics, and other companies are following suit. In late March, Colgate-Palmolive Co. announced a moratorium on testing, stating that 98 percent of testing could now be performed using available data or non-animal methodologies.

The numbers speak for themselves. According to the most recent figures on laboratory animals collected by the U.S. Department of Agriculture, the number of rabbits fell from almost 450,000 in 1973 to just 309,000 in 1997, a 31 percent decline. Whether driven by altruism, liability, federal enforcement or the bottom line, most companies see the need for safety testing. But safety testing can rarely be mentioned without bringing up the controversy surrounding the use of animals for those tests, and many companies label their products with statements indicating that no animals have been involved in testing.

"As far as we know," says Neil Wilcox, D.V.M., director of FDA's Office of Animal Care and Use, "what these companies do is use, for the most part, old reliable ingredients that have been proven safe [based on past animal data and a history of safe use] and then test the final product on people."

"There's kind of a fine point here," adds Gerald McEwan, Ph.D., vice president of science at the Cosmetic, Toiletry and Fragrance Association. "These companies that say they don't test on animals are skirting the issue. Practically every ingredient that's used in

cosmetics was at some point tested on animals. Probably a statement like, `no new animal testing' would be more accurate."

But what if a company wants to use a new ingredient?

Unlike drugs, FDA does not require pre-market approval for cosmetics. However, if a safety problem arises after a cosmetic has been marketed, FDA can take action to obtain the manufacturer's safety data on the product. Because there is not yet enough information on alternatives to animal testing to validate their use for ensuring human safety, FDA would, at this point, only accept animal safety tests.

According to the FDA, the Draize eye and skin irritancy tests continue to be considered among the most reliable methods currently available for evaluating the safety of a substance introduced into or around the eye or placed on the skin. Non-animal tests may be useful as screening tools to indicate the relative toxicity of a substance, however the responses and results of in vitro tests alone do not necessarily demonstrate the safety of a substance. The effects of a substance on a biochemical reaction or on a specific cell or tissue in culture may differ from its effect on a specific organ system as a whole.

Developed in 1944 by J.H. Draize, the basic testing procedures have remained unchanged, although some companies and individuals have made certain modifications to meet their own product requirements. The tests are used to determine whether chemicals or compounds, intended to come into contact with the eyes and skin, will cause irritation or injury. The tests were never intended to be exclusive means of determining the safety of any given product or chemical, but rather to be used in conjunction with other test and screening data.

The Eye Test involves a single dose of 0.1 ml, 100 mg, or 0.1 ml equivalent volume of the test substance, instilled into the conjunctival sac of one eye in as few as three test rabbits. The reaction is scored on days one, two and three, and again at one, two and three weeks. The Draize scale is used for rating ocular lesions, grading degree of corneal opacity, degree of corneal

involvement, iris condition and conjunctive redness, discharge and chemosis.

The Skin Test involves clipping small patches of fur from as few as three rabbits and applying 0.5 ml or 0.5 g of a test substance, which is held in place with gauze patches and non-irritating tape for 24 hours. After 24 hours, the patch is removed and the contact areas are examined for redness or swelling at 24 and 72 hours. Skin is evaluated on a scale of 1-8, ranging from no reaction to severe.

Alternatives Remain Elusive. There presently are no validated, non-animal alternatives for eye and skin irritation that can completely replace animal tests, according to federal safety officials. While some alternative test methods might be useful for screening purposes, they are not considered to be replacements for the current *in vivo* methods.

California lawmakers recently sought comments on legislation to ban animal testing for cosmetic and household products (Senate Bill No. 777), however the measure was pulled from the Senate Public Safety Committee shortly before a public hearing on the issue. The response of federal safety regulators to the proposal was unanimous -- Draize remains the last line of defense in testing eye and skin products, and to date there are no alternatives of equivalent efficacy.

In a March 19 letter on the proposal, Consumer Product Safety Commission associate director Mary Ann Danello, Ph.D., commented: "Although the Commission actively participates in and monitors progress in the area of alternatives to animal testing, at this time the staff does not believe that an adequate alternative exists for the Draize eye irritation test or other acute toxicity tests. It is our belief that some form of animal-based testing remains necessary for ensuring that consumer products ?contain proper precautionary labeling."

The Department of Health and Human Services and the Environmental Protection Agency also registered their concern. EPA's Steven Galson, M.D., M.P.H., director of the agency's office of science coordination and policy, wrote: "Passage of the

proposed legislation would be unfortunate, as it is based on false premises."

Galson rebutted virtually every argument made against the Draize test and the availability of non-animal alternatives. Regarding alternatives, he noted that high acidity or alkalinity can be used to screen chemicals so that they need not be tested in vivo. The European Centre for Validation of Alternative Methods (ECVAM) has concluded that the transcutaneous electrical resistance assay, SKIN and EPISKIN assays are valid alternatives (Fentem, J.H. et al, Toxicology in Vitro, p. 483-524) . In addition, a peer review panel for the Interagency Coordinating Committee on the Validation of Alternative methods concluded that Corrositex is an appropriate alternative to screen for acids, bases and acid derivatives. However, beyond these corrosivity tests there are no other alternatives for testing the safety of products for the eyes and skin, he stressed.

Other test methods might be valid for certain chemicals and products, but they do not apply across the board, and should not be considered equally valid to in vivo testing for regulatory purposes, Galson said. Industry often finds that it can use certain alternatives in-house to evaluate some specific product lines, and such uses should be encouraged.

"At the same time, the limitations of the alternatives beyond those product lines also need to be kept in mind. Unfortunately, we are not at a point where we can disregard the current in vivo methods to evaluate eye and skin irritation and corrosion. We can begin to use screens for corrosion potential in some cases, but that is the extent of our ability to replace the in vivo methods," he said.

John E. Bailey, Ph. D., director of FDA's cosmetics office, commented: "It is our strong belief that, although substantial progress has been made, some level of use of animal testing methods remains necessary for ensuring that cosmetic ingredients and products will not cause eye and skin irritation when used by consumers."

In an April 16 letter he stated, "It is the opinion of the scientific community and FDA that no validated tests currently exist that can completely replace animals in these evaluations."

"Draize may be impossible to replace with a single test," said Sidney Green, Ph.D., a toxicologist with FDA's Center for Food Safety and Applied Nutrition. He explained that because the Draize tests three different areas of the eye, replacing Draize will probably take a combination of tests, "but we've not seen that combination yet."

The cosmetics industry has taken one step toward database development - the Cosmetic Ingredient Review. Its purpose is to gather information from the scientific literature and from company files on the safety of cosmetic ingredients and make this information publicly available so that companies will know when effective non-animal testing exist.

In June 1998, a special workshop organized by ECVAM was held in the United Kingdom to examine possible Draize alternatives. An international panel of researchers and scientists reviewed a number of multilaboratory validation tests of alternatives. It concluded, "Continued use of the Draize test is not due to a shortage of potentially useful alternative methods, since more effort has probably been put into the development of alternatives to the Draize test than in seeking replacements to all other in vivo toxicity tests put together. However, no test, combination of tests, or testing strategy has yet been developed which meets all of the requirements of the regulatory authorities."

FDA's Wilcox explained that for FDA to approve any alternative, the test will have to produce results that can be reproduced in other laboratories. In addition, data bases will have to correlate historical animal test results with new lab results. "Database development and cooperation between FDA and industry is pivotal to the process," he notes.

FDA's division of toxicological review and evaluation is currently evaluating two alternatives to the Draize test. One is Eyetex, manufactured by Ropak Corp., of Irvine, Calif., a chemical assay that produces opacity similar to that of the animal cornea upon

exposure to irritants. The other is the use of vertebrate cell cultures from humans and mice. But until alternatives have been scientifically verified, the use of animal testing must be available for new ingredients and new products, said Wilcox.

附錄 二

Guidance on alternative appraisal methods for determining the eye irritation potential of cosmetic raw materials (Japanese)

1. Introduction

Information on eye irritation potential is one of the required components that must be submitted with the application for the approval of cosmetic products containing new raw materials which have not previously been approved for use in cosmetic products. This is usually obtained by the Draize test, a test that uses rabbits. However, due to concerns over animal welfare, it has been suggested that alternative methods may be used if they are proved to be appropriate as substitutes for the method presently employed.

Several in vitro methods have been examined, of which some have correlated well with the results of the Draize test. However, non one test has been able to reliably predict the results of Draize test over the full range of test substances. On the other hand, the results of inter-laboratory validation studies have suggested that some of these alternative methods can identify either non-irritants or strong irritants, or both.

This guidance describes a scheme that uses alternative methods in combination with the Draize test in order to reduce the number of animals required for testing and minimize the suffering of the animals without lowering the reliability of evaluation of the eye irritation potential of cosmetic raw materials. Alternative methods constitute only a part of the evaluation scheme. This is because experience of the utilization of those methods in actual situation seemed insufficient. As further test results are accumulated by testing many cosmetic raw materials by both the Draize test method and alternative methods in accordance with this guidance, revisions to the guidance may be necessary.

The appraisal scheme outlined in this guidance does not

necessarily require the same procedure for every substance or at all testing facilities. The emphasis is on selecting the appropriate method according to the purpose of the test, properties of the test substance, experience and equipment available in the testing facilities, etc., while taking into consideration the following points. In some situations, the appraisal may only be conducted by the Draize test.

2. Points to be considered in the appraisal of eye irritation potential of cosmetic raw materials

As cosmetics are used in daily by ordinary people, safety of the products is of great importance. Specifically, cosmetics must demonstrate little or no adverse effects. Most of the cosmetic raw materials are non- or weak irritants, but some of them demonstrate significant irritancy. For the latter group, it is necessary to establish the safe concentration range for their use in cosmetics. Cosmetics are not intended for use in the eye. The risk of injury due to accidental contact with the eye can be minimized by appropriate treatment such as rinsing the eye.

3. Choice of alternative methods for evaluating eye irritation potential

The range of substances that can be examined by alternative methods and the reliability of the appraisal depend on the mechanism upon which the alternative methods are based and on characteristics such as the sensitivity, reproducibility, correlation of the results with those of the Draize test. Therefore, the method to be employed must be one that has been evaluated objectively to characterize the above mentioned properties by appropriate validation. Proper appraisal of a test substance is possible if the method is appropriately chosen according to the physical/chemical properties and the degree of irritation potential of the test substance, as well as other toxicological information obtained in advance.

A scheme to appraise eye irritation potential should consist of

three stages. In the first stage, the decision to conducted the appraisal by alternative methods or by the Draize test is made according to the physical/chemical properties of the test substance. When alternative methods are used, the non- and strong irritants are identified in the second stage. Approximate appraisal of the degree of irritation potential of the irritants is also made for the other substances. In the third stage, the irritation potential is appraised using animals for those substances which cannot be judged as non-irritants. Information regarding the physical/chemical properties of the test substance is also necessary for the appraisal of data obtained at the second stage. Depending on the equipment and experience available in the testing facility, the second stage may be omitted.

Animal tests at the third stage must be conducted in a manner that minimizes the suffering of animals. This may be accomplished by diluting the test substance based on the concentration to be formulated in the products and the results of in vitro tests.

4. Available alternative methods and points to be considered

A test method for which the applicable range, sensitivity, reproducibility and correlation with the Draize test for appropriate test substances that have been determined through validation should be used. As an example of such a method, a cytotoxicity test method may be used to examine the influence of test substances on the viability and proliferation of cultured cells, affording results such as 50% inhibitory concentration, IC50, etc. (Note 1)

5. Scheme of Appraisal

First stage:

Determine whether the test substance can be appraised by alternative methods or by animal test on the basis of the physical/chemical properties such as chemical structure, pH, acidity, alkalinity. If applicable, select appropriate alternative

methods. (Notes 2-5)

Second stage:

Along with appraisal of approximate irritation potential, establish whether the test substance can be classified as a non-irritant (Maximum Average Score (MAS) in the eye irritation test is $0 \leqq$ and $\leqq 5$) using only alternative methods.

Third stage:

If the test substance has not been established as a non-irritant, appraise the irritation potential of the test substance using the animal test. At this stage, take measures to minimize the suffering of animals, taking into account the concentration of the substance to be formulated in the cosmetics. If the test substance is expected to be at least moderately-irritant from information obtained in advance and/or the test results of the alternative methods, consider diluting the test substance taking into account factors such as the purpose of the test.

6. References

1) Guidebook on application for the manufacturing of cosmetics and quasi-drugs, Third Ed., Supervised by the Pharmaceuticals and Cosmetics Division, Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, Yakuji Nippo Co., Ltd., 1996

Note 1)
Methods using cell lines derived from rabbit cornea (SIRC cells), human uterus carcinoma (HeLa cells), etc. in culture medium supplemented with serum generally provide high sensitivity and good reproducibility. These results show a relatively good correlation with the results of the Draize test. Using an appropriate combination of these methods, identification and classification as an irritant or non-irritant, and an approximate appraisal of the degree of irritating potential are possible. Among other cytotoxicity tests and artificial skin models, there are methods that can be used for identification and classification of irritants and non-irritants.

Note 2)
A test substance classified either as a strong acid or a strong alkali with a pH of below 2.0 or above 11.5, respectively, is generally considered to be a strong irritant when their acidity or alkalinity are high.

Note 3)
A test substance showing strong irritancy or corrosive action on the skin is also generally considered to be a strong irritant.

Note 4)
If a test substance cannot be uniformly mixed with the culture medium in the cytotoxicity tests, the results obtained may not properly reflect its cytotoxicity.

Note 5)
The applicability of cytotoxicity tests has not been confirmed for test substances showing strongly acidic or alkaline characteristics, or for volatile substances such as alcohol.

Note 6)
A threshold that is employed for identification of non-irritants based only on the results of a cytotoxicity test should be set at a value that minimize the risk of false-negative results. This value should be higher than the concentration at which the test substance can be regarded as non-irritant under any experimental conditions (for example, when the IC50, in the cytotoxicity test with the culture medium containing serum is higher than 5000 (g/ml) or higher than the value obtained by multiplying by an ample safety factor, the IC50 value of a standard substance that has been clearly established as a non-irritant.

Note 7)
Among the alternative methods, there are some such as cytotoxicity test, in which the IC 50 value is greatly influenced by the kind of cell line used and the culture conditions. In such cases, it is desirable to appraise the validity of the test results by

comparing the results with those of several standard substances, including both negative and positive reference substances.

Note 8)
A 10% polyoxyethylene sorbitan monolaurate (20 E.O.) (Tween 20) solution is used as a reference substance for non-irritancy in the appraisal of cytotoxicity of the test substance, and 10% polyoxyethylene octylphenylether (10 E.O.) (Triton X-100) and 10% sodium lauryl sulfate (SLS) solutions are used as positive reference substances. Appropriate substances are selected according to the characteristics of the test method and tested at the same time as the test substance.
The irritation potential of substances that cannot be judged as non irritant based only on the alternative methods are evaluated as follows by comparison with standard and positive reference substances.
Practically non-irritant : Substances with a higher IC50 than that of Tween 20 (MAS around 0)
Slight irritant : Substances with IC50 lower than that of Tween 20 and higher than that of SLS (MAS around 30)
Moderate irritant : Substances with IC50 lower than that of SLS and higher than that of Triton X-100 (MAS around 50)
Strong irritant : Substances with a lower IC50 than that of Triton X-100

Note 9)
If a test substance is found to be a non-irritant on the basis of alternative methods alone and will not be formulated in the products at a concentration in excess of 10%, it may be appraised as a non-irritant without animal tests. Animal tests are necessary for substances which do not meet the above conditions.

http://hayato.med.osaka-u.ac.jp/index/societies-j/alt/guidance-e.html